UNIVERSITY OF ALBERTA

CHEMICAL INACTIVATION OF CRYPTOSPORIDIUM PARVUM
IN WATER

by

HANBIN LI ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of DOCTOR OF PHILOSOPHY
in
ENVIRONMENTAL ENGINEERING

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

EDMONTON, ALBERTA
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TITLE OF THESIS: CHEMICAL INACTIVATION OF CRYPTOSPORIDIUM PARVUM IN WATER

DEGREE FOR WHICH THIS THESIS WAS GRANTED: DOCTOR OF PHILOSOPHY

YEAR THIS DEGREE WAS GRANTED: 2001

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Dated: April 4, 2001

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled CHEMICAL INACTIVATION OF CRYPTOSPORIDIUM PARVUM IN WATER submitted by HANBIN LI in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ENVIRONMENTAL ENGINEERING.

Daniel W. Smith

Dr. D. W. Smith - Supervisor

Dr. M. Belosevic - Co-supervisor

Ian Buchanan

Dr. I. D. Buchanan

A Robinson

Dr. A. Robinson

P. Fedorak

Dr. P. M. Fedorak

Dr. G. Gordon - External Examiner

Dated: 4 april 2001

DEDICATION

To my parents and my brother Hanzhao

for their love and support

ABSTRACT

Inactivation of *Cryptosporidium parvum* oocysts in water using single or sequential microorganism reduction chemicals was studied. Experiments were conducted at bench-scale in oxidant demand-free 0.05 M phosphate buffer. Animal infectivity using neonatal CD-1 mice was used for evaluation of oocyst infectivity following treatment. Inactivation kinetics were determined for single treatment using ozone, chlorine dioxide, free chlorine or monochloramine, and sequential treatment using ozone followed by free chlorine or monochloramine.

Survival curves for the *C. parvum* oocysts after ozone treatment were characterized by an initial lag and a slight tail-off at high levels of treatment. For chlorine dioxide, free chlorine and monochloramine treatment, the survival of the oocysts declined linearly with the $C_{avg}t$ (arithmetic average of initial and final concentration × contact time) products. Temperature was a critical factor for *C. parvum* oocyst inactivation and the inactivation rates decreased dramatically at low temperatures. Water pH was not a significant factor affecting the inactivation rate constant of ozone (pH 6 to 8) or chlorine dioxide (pH 6 to 11). A non-linear Incomplete gamma Hom (I.g.H.) model with temperature correction was developed for ozone inactivation, and Chick-Watson models with temperature correction were developed for chlorine dioxide, free chlorine and monochloramine inactivation. Between 1 and 37°C, every 10°C drop of the water temperature resulted in the reaction rate constant decreasing by a factor of 2.2 and 2.3 for

ozone and chlorine dioxide, respectively, corresponding to activation energies of 52 and 55 kJ/mol.

Evident synergy was observed for sequential inactivation using ozone followed by free chlorine or monochloramine. Factors that affected sequential inactivation included the level of ozone primary treatment, and the $C_{avg}t$ product of the secondary treatment and water temperature. Gross kills of the sequential treatment were greater for higher levels of ozone pre-treatment and increased linearly with the $C_{avg}t$ products of the secondary treatment. High water temperature was favorable for both gross kill and inactivation synergy. For 1.6 log-units of ozone primary kill, the efficacy of free chlorine or monochloramine secondary treatment was comparable, and their reaction rate constant increased by a factor of about 1.7 for 10°C increase in water temperature.

ACKNOWLEDGEMENTS

This work was initially supervised by Dr. Gordon R. Finch. Since Dr. Finch's untimely death in January 2000, Dr. Daniel W. Smith continued the supervisory role.

I wish to thank my supervisors Drs. Finch and Smith and co-supervisor Dr. Miodrag Belosevic for their invaluable guidance and support.

I wish to thank the members of my committee for their insightful comments and questions which made for a better thesis.

The technical support and assistance provided by Mr. C. Kucharski and Ms. S. Lebvere was greatly appreciated.

The American Water Works Association Research Foundation and the U.S. Environmental Protection Agency provided primary funding for this project with additional support from the Chemical Manufacturers Association. The Natural Sciences and Engineering Research Council of Canada provided partial funding for this research through research and equipment grants to Drs. Finch, Belosevic and Smith.

CONTENTS

CHAPTER 1 INTRODUCTION 1

1.1 Overview 1

1.1.1 The Significance of Microorganism Reduction 1

1.1.2 Protozoan Pathogens in Water 2

1.1.3 Current State of *Cryptosporidium* spp. Microorganism Reduction 3

1.2 Goals and Objectives 4

1.3 Scope 5

1.4 Outline of the Thesis 6

CHAPTER 2 LITERATURE REVIEW 7

2.1 *Cryptosporidium parvum* 7

2.1.1 Biology 7

2.1.2 Occurrence and Detection 9

2.1.3 Oocyst Viability Assessment 10

2.2 Water Microorganism Reduction 12

2.2.1 Control of *C. parvum* in Water 12

2.2.2 Microbial Reduction Chemicals 14

2.2.3 Factors in Microorganism Reduction 20

2.2.4 Disinfection By-Products (DBPs) 23

2.3 Chemical Inactivation of *C. parvum* Oocysts 24

2.3.1 Single Chemicals 24

2.3.2 Multiple Chemicals 27

CHAPTER 3 MATERIALS AND METHODS 30
3.1 Parasitology Methods 30
3.1.1 Biosafety 30
3.1.2 Production of *C. parvum* Oocysts 30
3.1.3 Animal Infectivity Assay 32
3.2 Oxidants and Apparatus 35
3.2.1 Chlorine 35
3.2.2 Monochloramine 36
3.2.3 Ozone 36
3.2.4 Chlorine Dioxide 37
3.2.5 Oxidant Demand-Free Glassware 38
3.2.6 Reactor Vessels 39
3.2.7 Temperature Control 40
3.3 Determination of Oxidants Concentrations 40
3.3.1 Ozone 40
3.3.2 Chlorine Dioxide 41
3.3.3 Chlorine and Chloramine 42
3.4 Water Type 43
3.5 Microorganism Reduction Procedure 44
3.5.1 Single Oxidants 45
3.5.2 Sequential Oxidants 46
3.6 Kinetic Modeling 47
3.6.1 Chick-Watson Model and I.g.H. Model 47
3.6.2 Temperature Corrected Models 49
3.6.3 Empirical Model for Sequential Inactivation 51
3.7 Experimental Design 52
3.7.1 Single Treatment 52

3.7.2 Sequential Treatment 53

3.8 Statistics 54

3.8.1 Maximum-Likelihood Model Parameters Estimation 54

3.8.2 Dose-Response Model Parameter Estimation 56

3.8.3 Marginal Confidence and Joined Confidence Limits 56

3.8.4 Confidence Band for Linear Model 57

3.8.5 Confidence Band for Nonlinear Model 58

3.8.6 Inverse Prediction for Design Criteria Development 60

3.9 Solving I.g.H. models in MS Excel 62

3.9.1 Calculation of Log-kill 62

3.9.2 Calculation of Required Initial Concentration (C_o) 63

3.9.3 Calculation of Required Contact Time (t) 64

CHAPTER 4 DOSE-RESPONSE AND CONTROLS 65

4.1 Dose-Response 65

4.2 Control 68

CHAPTER 5 OZONE 70

5.1 Introduction 70

5.2 Experimental Settings 70

5.3 Results and Discussion 71

5.3.1 Microbial Reduction Following Ozone Treatment 71

5.3.2 Microorganism Reduction Kinetics 75

5.3.3 pH Effect 78

5.3.4 Temperature Effect 81

5.4 Ozone Disinfection Design Criteria 82

5.5 Summary 89

CHAPTER 6 CHLORINE DIOXIDE 91

6.1 Introduction 91

6.2 Experimental Settings 91

6.3 Results and Discussion 92

6.3.1 Microbial Reduction Following Chorine Dioxide Treatment 92

6.3.2 Inactivation Kinetics 96

6.3.3 pH Effect 102

6.3.4 Temperature Effect 103

6.3.5 Chlorine Dioxide Disinfection Design Criteria 105

6.4 Summary 110

CHAPTER 7 CHLORINE SPECIES 112

7.1 Introduction 112

7.2 Experimental Settings 113

7.3 Results with Free Chlorine 113

7.3.1 Microorganism Reduction After Free Chlorine Treatment 113

7.3.2 Kinetic Modeling 115

7.3.3 Temperature Effect 118

7.4 Results with Monochloramine 119

7.4.1 Microorganism Reduction After Monochloramine Treatment 119

7.4.2 Kinetic Modeling 121

7.4.3 Temperature Effect 122

7.5 Summary 123

CHAPTER 8 OZONE FOLLOWED BY FREE CHLORINE 125

8.1 Introduction 125

8.2 Experimental Settings 125

8.3 Results of Micoorganism Reduction After Treatment 126

8.3.1 At 1°C 126
8.3.2 At 10°C 129
8.3.3 At 22°C 131
8.4 Chick-Watson Models 133
8.5 Effect of Ozone Pre-treatment 137
8.6 Effect of Temperature 138
8.7 Hypothesis of Synergy 138
8.8 Summary 141
CHAPTER 9 OZONE FOLLOWED BY MONOCHLORAMINE 142
9.1 Introduction 142
9.2 Experimental Settings 142
9.3 Results of Microorganism Reduction After Treatment 143
9.3.1 At 1°C 143
9.3.2 At 10°C 145
9.3.3 At 22°C 147
9.4 Chick-Watson Models 150
9.5 Effect of Ozone Pre-treatment 151
9.6 Effect of Temperature 154
9.7 Discussion 154
9.8 Summary 155
CHAPTER 10 GENERAL DISCUSSION 157
10.1 Summary of the Findings 157
10.2 Application to Natural Water 161
CHAPTER 11 CONCLUSIONS AND RECOMMENDATIONS 165
11.1 Conclusions 165
11.2 Recommendations 168

REFERENCE 169

APPENDIX A CONCENTRATION AND CONTACT TIME PROFILE FOR EXPERIMENTAL TRIALS 197

APPENDIX B DOSE-RESPONSE DATA 199

APPENDIX C SUMMARY OF CONTROL TRIALS 203

APPENDIX D ANIMAL DATA FROM EXPERIMENTAL AND CONTROL TRIALS 206

LIST OF TABLES

Table 4-1. Logistic dose-response models for neonatal CD-1 mice exposed to different batches of *C. parvum* oocysts 66

Table 5-1. Summary of ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 at 1±0.5°C 72

Table 5-2. Summary of ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 at 22±1°C 73

Table 5-3. Summary of ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 7 at 5, 13 and 37°C 74

Table 5-4. Model parameters for ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 and 1 to 37°C 77

Table 5-5. Activation energies for ozone inactivation of microorganisms 82

Table 5-6. Ct requirement for ozone inactivation of *C. parvum* oocysts at pH 6 to 8, given by I.g.H. model, assuming a constant ozone residual (no safety factor) 85

Table 6-1. Summary of chorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 11 and 22±1°C 93

Table 6-2. Summary of chorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 11 and 1±0.5°C 95

Table 6-3. Summary of chlorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 5, 13 and 37 °C 96

Table 6-4. Model parameters for chlorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 and 1 to 37°C 99

Table 6-5. Activation energies for chlorine dioxide inactivation of microorganisms 104

Table 6-6. Ct requirement for chlorine dioxide inactivation of *C. parvum* oocysts at pH 6 to 11 given by the Chick-Watson model, assuming constant chlorine dioxide residual in oxidant demand-free 0.05 M phosphate buffer (no safety factor) 105

Table 7-1. Summary of free chlorine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1 to 22 °C 114

Table 7-2. Chick-Watson model parameters of free chlorine or monochloramine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at 1 to 22°C 116

Table 7-3. Summary of monochloramine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1 to 22°C 120

Table 8-1. Summary of *C. parvum* oocyst inactivation using ozone followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1°C 127

Table 8-2. Summary of *C. parvum* oocyst inactivation using ozone followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 10°C 129

Table 8-3. Summary of *C. parvum* oocyst inactivation using ozone followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 22°C 132

Table 8-4. Chick-Watson model parameters for sequential inactivation of *C. parvum* oocysts using ozone followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1 to 22°C 136

Table 9-1. Summary of *C. parvum* oocyst inactivation using ozone followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1°C 144

Table 9-2. Summary of *C. parvum* oocyst inactivation using ozone followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 10°C 146

Table 9-3. Summary of *C. parvum* oocyst inactivation using ozone followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 22°C 148

Table 9-4. Chick-Watson model parameters for sequential inactivation of *C. parvum* oocysts using ozone followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1 to 22°C 151

Table B-1. Dose-response data of neonatal CD-1 mouse to *C. parvum* oocysts (batch # 1 to 7) 200

Table B-2. Dose-response data of neonatal CD-1 mouse to *C. parvum* oocysts (batch # 8 to 14) 201

Table B-3. Dose-response data of neonatal CD-1 mouse to *C. parvum* oocysts (batch # 15 to 19) 202

Table C-1. Control trials of *C. parvum* inactivation tests in oxidant demand-free 0.05 M phosphate buffer solution 204

Table D-1. Animal data of viability assay using neonatal CD-1 mouse for experimental and control trials 207

LIST OF FIGURES

Figure 2-1. Life cycle of *Cryptosporidium parvum* 8

Figure 3-1. Temperature coefficient as a function of the range of temperature used for its estimation ($T_1 = 0°C$) 50

Figure 4-1. Model prediction with 90% confidence interval for CD-1 mice dose-response to *C. parvum* oocysts (batch No. 15) 67

Figure 4-2. 90% Joint confidence interval (JCR) of CD-1 mice dose-response model parameters, β_o and β_1, for batch No. 15 *C. parvum* oocysts 68

Figure 4-3. Histogram of the reduced infectivity of *C. parvum* oocysts in the control trials in oxidant demand-free 0.05 M phosphate buffer 69

Figure 5-1. Survival ratios of *C. parvum* oocysts as the function of $C_{avg}t$ products after ozone treatment in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 and 1 to 37°C 76

Figure 5-2. Temperature corrected I.g.H. model fit for ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 and 1 to 37°C: a) as a function of temperature, and b) as a function of pH 79

Figure 5-3. Ozone dose and contact time for 1.0 log-unit inactivation of *C. parvum* oocysts predicted by I.g.H. model at pH 6 to 8 and 1 to 22°C, assuming first-order ozone disappearance rate constants of 0.05 1/min and buffered oxidant demand-free water 83

Figure 5-4. Ozone dose the contact time for 2.0 log-units inactivation of *C. parvum* oocysts predicted by I.g.H. model at pH 6 to 8 and 1 to 22°C, assuming first-order ozone disappearance rate constants of 0.05 1/min and buffered oxidant demand-free water 84

Figure 5-5. I.g.H. model predicted survival curves of *C. parvum* oocysts under 1.5 mg/L initial ozone treatment at different temperatures, assuming first-order ozone disappearance rate constants of 0.05 1/min and buffered oxidant demand-free water 87

Figure 5-6. 1.0 log-unit design curves with 90% confidence level for ozone inactivation of *C. parvum* oocysts at pH 6 to 8 and 1 to 22°C given by I.g.H. modal, assuming first-order ozone disappearance rate constant of 0.05 1/min and buffered oxidant demand-free water 88

Figure 5-7. 2.0 log-units design curves with 90% confidence level for ozone inactivation of *C. parvum* oocysts at pH 6 to 8 and 1 to 22°C given by I.g.H. modal, assuming first-order ozone disappearance rate constant of 0.05 1/min and buffered oxidant demand-free water 89

Figure 6-1. Survival ratios of *C. parvum* oocysts as the function of $C_{avg}t$ products after chlorine dioxide treatment in oxidant demand-free 0.05 M phosphate buffer at temperature from 1 to 37°C (the lines across the origin are the best fit curves at different temperatures) 97

Figure 6-2. Temperature corrected Chick-Watson model fit for chlorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 11 and 1 to 37°C: a) as a function of temperature, and b) as a function of pH 100

Figure 6-3. 0.5 log-units design curves with 90% confidence level for chlorine dioxide inactivation of *C. parvum* oocysts at pH 6 to 11 and 1 to 22°C given by Chick-Watson model, assuming first-order chlorine dioxide disappearance rate constant of 0.001 1/min (using oxidant demand-free 0.05 M phosphate buffer) 107

Figure 6-4. 1.0 log-unit design curves with 90% confidence level for chlorine dioxide inactivation of *C. parvum* oocysts at pH 6 to 11 and 1 to 22°C given by Chick-Watson model, assuming first-order chlorine dioxide disappearance rate constant of 0.001 1/min (using oxidant demand-free 0.05 M phosphate buffer) 108

Figure 6-5. 2.0 log-units design curves with 90% confidence level for chlorine dioxide inactivation of *C. parvum* oocysts at pH 6 to 11 and 1 to 22°C given by Chick-Watson model, assuming first-order chlorine dioxide disappearance rate constant of 0.001 1/min (using oxidant demand-free 0.05 M phosphate buffer) 109

Figure 7-1. Temperature corrected Chick-Watson model fit for free chlorine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1 to 22°C 117

Figure 7-2. Temperature corrected Chick-Watson model fit for monochloramine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1 to 22°C 122

Figure 8-1. Survival ratios of *C. parvum* oocysts as a function of free chlorine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1°C 128

Figure 8-2. Survival ratios of *C. parvum* oocysts as a function of free chlorine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 10°C 130

Figure 8-3. Survival ratios of *C. parvum* oocysts as a function of free chlorine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 22°C 133

Figure 8-4. Chick-Watson model prediction on inactivation of *C. parvum* oocysts using 0.4 log-units of ozone pre-treatment kill followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1, 10 and 22°C 134

Figure 8-5. Chick-Watson model prediction on inactivation of *C. parvum* oocysts using 1.6 log-units of ozone pre-treatment kill followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1, 10 and 22°C 135

Figure 9-1. Survival ratios of *C. parvum* oocysts as a function of monochloramine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1°C 145

Figure 9-2. Survival ratios of *C. parvum* oocysts as a function of monochloramine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 10°C 147

Figure 9-3. Survival ratios of *C. parvum* oocysts as a function of monochloramine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 8 at 22°C 149

Figure 9-4. Chick-Watson model prediction on inactivation of *C. parvum* oocysts using 0.4 log-units of ozone pre-treatment kill followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1, 10 and 22°C 152

Figure 9-5. Chick-Watson model prediction on inactivation of *C. parvum* oocysts using 1.4 log-units of ozone pre-treatment kill followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1, 10 and 22°C 153

Figure A-1. Ozone residual concentration *versus* time profile for trial No. 611 (pH 8.0 and 22°C) 198

LIST OF SYMBOLS

The following symbols and terms are used:

A = Arrhenius pre-exponential factor, also termed frequency factor (1/min)
°C = degrees Celsius
C = oxidant concentration (mg/L)
C_{avg} = arithmetic average of C_o and C_f, $C_{avg} = (C_f + C_o)/2$ (mg/L)
C_f = oxidant residual measured at the end of the contact time t (mg/L)
C_o = oxidant residual measured at time zero (mg/L)
Cl_2 = free chlorine
ClO_2 = chlorine dioxide
DPBs = disinfection by-products
DPD = N, N-diethyl-p-phenylenediamine
E_a = van't Hoff-Arrhenius activation energy (J/mol)
E_0 = standard oxidation-reduction potential (V)
$\hat{F}$ = Jacobian matrix at $\hat{\beta}$
$F^{\alpha}_{(1,n-p)}$ = upper α quantile of Fisher's distribution
HAAs = Haloacetic acids
h = hour
ID_{50} = infectious dose for 50% of infection in the cohort (oocysts per animal)
I_r = inactivation ratio (log-unit)
I_{r1} = inactivation ratio of the primary treatment (log-unit)

I_{r2} = inactivation ratio caused by the secondary chemicals when it is used singly (log-unit)

I'_{r2} = inactivation ratio caused by the secondary treatment (log-unit)

j = number of data points used in model parameter estimation

k = inactivation rate constant (L/(mg·min) for linear model)

k_T = inactivation rate constant for a specified temperature T (L/(mg·min) for linear model)

$\hat{k}$ = optimal k parameter estimate (L/(mg·min) for linear model)

k' = first-order chemical disinfectant disappearance rate constant (1/min)

L_o = likelihood function for normally distributed errors

L = litre

m = empirical kinetic model parameter

$\hat{m}$ = optimal m parameter estimate

MCL = maximum contaminant level

mg/L = milligram per litre

mg·min/L = concentration (mg/L) and time (min) product

min = minute

N = number of surviving oocysts at time t

N_o = number of oocysts at time zero

n = number of live oocysts after treatment

n_o = number of live oocysts in the control

n = empirical kinetic model parameter

$\hat{n}$ = optimal n parameter estimate

n_d = number of mice used for dose-response model estimation for that batch of oocysts

NH_2Cl = monochloramine

O_3 = ozone

P = proportion of mice in a cohort scoring positive for a given inoculum X

p = number of model parameters

R = universal gas constant, 8.31 J/(mol.K)

s = square root of the model variance

S = variance of model estimation

SWTR = Surface Water Treatment Rule

T = temperature in Kelvins (K) for the van't Hoff-Arrhenius model and °C for the disinfection kinetic models

t = contact time (min)

t_{10} = minimum exposure time for 90% of the reactor contents

US EPA = United States Environmental Protection Agency

THMs = trihalomethanes

X = oocyst inoculum given to each neonate CD-1 mouse

$\mathbf{x}$ = matrix of the disinfection condition of the data set

x' = empirical kinetic model parameter

y_i = observed value for trial i^{th}

Y_i = positive (Y_i=1) or negative (Y_i=0) infection of the mice

$Z_{\alpha/2}$ = normal distribution

α = level of statistical significance

β = matrix of model parameters: for the logistic response model, refers to β_o and β_1; for the temperature corrected I.g.H. model, refers to θ, k, m, and n; for the temperature corrected Chick-Watson, refers to θ, k and n

$\hat{\beta}$ = optimal model prediction of matrix β

γ = incomplete gamma function

θ = temperature coefficient

$\hat{\theta}$ = optimal θ parameter estimate

μ_i = model predicted kill for trial i^{th}

ν_o = the number of non-censored data points

ν_1 = the number of left-censored data points (less than)

ν_2 = the number of right-censored data points (great than)

π' = logistic mean response

σ = square root of the regression error variance for entire population

$\hat{\Sigma}$ = variance-covariance matrix

$\Phi(z)$ = cumulative standard normal distribution

χ^2 = Chi-squared distribution

CHAPTER 1

INTRODUCTION

1.1 OVERVIEW

1.1.1 The Significance of Microorganism Reduction

In North America, waterborne diseases were a serious health problem until the turn of this century. The introduction of filtration for removal of particles and application of liquid chlorine to the filtered water, starting in 1904 in the US (White 1972), marked the beginning of pathogen control in drinking water treatment. Since then, epidemic outbreaks of waterborne diseases that cause major mortality, such as cholera and typhoid, have been decreased dramatically in the communities with chlorinated water supply.

In the 1970s, two issues related to water chlorination initiated the re-evaluation of the water treatment systems. The first issue was that the disinfection by-products (DPBs), chloroform and trihalomethanes (THMs), may be carcinogenic (Bull et al. 1995). These halogenated by-products are formed when the microorganism reduction chemicals react with the organic compounds in water. The other issue was that some newly recognized waterborne pathogens including types of viruses, species of bacteria and protozoans were very resistant to the conventional chlorination (Harms and Long 1988; Knochel 1991; West 1991). Among them, some protozoan pathogens, *Giardia* spp. and

Cryptosporidium spp., were of most concerned due to the frequent outbreaks of the diseases they cause.

1.1.2 Protozoan Pathogens in Water

Cryptosporidium spp. have been recognized as a frequent cause of waterborne disease (D'Antonio et al. 1985; Hayes et al. 1989; Rush et al. 1990; Pett et al. 1993). The occurrence of *Cryptosporidium* spp. oocysts has been reported in surface and ground waters in different geological locations (Rose 1988; 1990; LeChevallier et al. 1991a; Lisle and Rose 1995; Hancock et al. 1998). The dormant forms of *Cryptosporidium* spp. oocysts are very resistant to the water microorganism reduction treatment (Goldstein et al. 1996; Steiner et al. 1997). Ingestion of *Cryptosporidium* spp. oocysts might cause severe diahrrea, which can be fatal to children and immunosuppressed persons (Chui and Owen 1994; Colford et al. 1996; Gerba et al. 1996). At present, there is no safe and effective chemotherapeutic treatment for cryptosporidiosis.

Under the Surface Water Treatment Rule (SWTR), the US EPA requires 3.0 log-units removal of *Giardia* spp. cysts from the surface water supplies. In the Interim Enhanced Surface Water Treatment Rule (IESWTR) (U.S. Environmental Protection Agency 1998b), a maximum contaminant level goal (MCLG) of zero for *Cryptosporidium* spp. was recommended. A 2 log-unit of *Cryptosporidium* spp. removal was required for the filter systems in water treatment plants that serve 10,000 or more people and use surface water or groundwater under the direct influence of surface water

(GWUDI) as the water source.

1.1.3 Current State of *Cryptosporidium* spp. Microorganism Reduction

The conventional microorganism reduction chemicals in water treatment, free chlorine or combined chlorines, have been shown not to be very effective for inactivation of *Cryptosporidium parvum* oocysts (Korich et al. 1990b). In contrast, the results of chemical inactivation of *C. parvum* under bench-scale conditions (Peeters et al. 1989; Korich et al. 1990b; Perrine et al. 1990; Parker et al. 1993; Ransome et al. 1993; Finch et al. 1994b), have indicated that ozone and chlorine dioxide were the most effective chemicals for control of *C. parvum* oocysts in drinking water. In the last few years, data were collected in our laboratory for ozone or chlorine dioxide inactivation of *C. parvum* oocysts, using experimental protocols that were amenable to rigorous analysis of inactivation kinetics and the simultaneous disappearance of the chemicals (Liyanage 1998; Gyürék et al. 1999). However, most of the studies were conducted at room temperature and the effects of temperature and pH were not examined.

Studies of sequential treatment showed that some chemical combinations were very effective for inactivation of *C. parvum* oocysts (Finch et al. 1994a; Gyürék 1997). In water treatment facilities that use ozone for the primary disinfection, free chlorine or monochloramine is frequently added as the secondary disinfectant. The preliminary screening experiments from an AWWA Research Foundation project (Finch et al. 2000) showed that pre-treatment using ozone or chlorine dioxide significantly increased the efficacy of free chlorine or monochloramine. In this study, the most promising combinations of the microorganism reduction chemicals were examined. Furthermore, the factors relative to disinfection synergy were investigated and quantified.

1.2 GOALS AND OBJECTIVES

This study was undertaken to assess the inactivation of *C. parvum* oocysts using single or combined microorganism reduction chemicals under a variety of conditions. For the single treatment processes, ozone, chlorine dioxide, free chlorine and monochloramine were investigated. In the sequential treatment processes, ozone followed by free chlorine or monochloramine were examined. The goal of this study was to determine the microorganism reduction kinetics of these chemicals used singly or in combination, and to identify the important factors in design and operation of these processes for *C. parvum* inactivation.

To achieve the study goal, the following research objectives were established:

1. Determine the effect of water temperature and pH in ozone inactivation of *C. parvum* oocysts. This study focused on the ozone performance at low temperature, and pH values from 6 to 8. The results of this study were combined with the room temperature results from previous studies (Gyürék et al. 1999) to generate a kinetic model for temperature 1 to 37°C and pH values from 6 to 8.
2. Determine the effect of water temperature and pH in chlorine dioxide

inactivation of *C. parvum* oocysts. Chorine dioxide inactivation was studied from 1 to 37°C and pH values from 6 to 11. A kinetic model covering these temperature and pH conditions was developed.

3. Determine the influence of temperature on the inactivation of *C. parvum* oocysts using free chlorine or monochloramine. Kinetic models with temperature correction were developed for free chlorine at pH 6 and for monochloramine at pH 8, temperatures from 1 to 22°C.
4. Determine the factors relative to sequential inactivation of *C. parvum* oocysts using ozone followed by free chlorine at pH 6 and temperatures from 1 to 22°C. The factors that were investigated included the level of ozone pre-treatment, free chlorine Ct (concentration × contact time) product and water temperature. Empirical models for ozone followed by free chlorine sequential inactivation were developed.
5. Determine the factors relative to sequential inactivation of *C. parvum* oocysts using ozone followed by monochloramine at pH 8 and temperatures from 1 to 22°C. The factors that were investigated included the level of ozone pre-treatment, monochloramine Ct product and water temperature. Empirical models for ozone followed by monochloramine inactivation were developed.

1.3 SCOPE

The data in this study were collected under strictly controlled bench-scale experiments in laboratory water. Laboratory water was used as the principal water type. By using a reproducible water for all experiments, sources of error due to batch variation in other types of water were reduced. Animal infectivity was used to measure oocyst viability before and after treatment, because animal infectivity is the more accurate and reliable method for evaluating of the viability of *C. parvum* oocysts.

1.4 OUTLINE OF THE THESIS

The dissertation was organized following the "traditional" format. The literature review (Chapter 2) and the materials and methods (Chapter 3) were put in separated chapters. The results and discussion included six chapters: the dose-response and controls (Chapter 4), single chemical treatment of *C. parvum* oocysts using ozone (Chapter 5), chlorine dioxide (Chapter 6) and chlorine species (Chapter 7), and the sequential treatment using ozone followed by free chlorine (Chapter 8) or monochloramine (Chapter 9). After the results and discussion, the thesis was finalized by the general discussion (Chapter 10) and the conclusions and recommendation (Chapter 11). In the appendices, example of the oxidant concentration in the reactor *versus* time profile (Appendix A), animal data of CD-1 mice dose-response model (Appendix B), control trials (Appendix C), and infectivity data of all the trials (Appendix D) were included.

CHAPTER 2

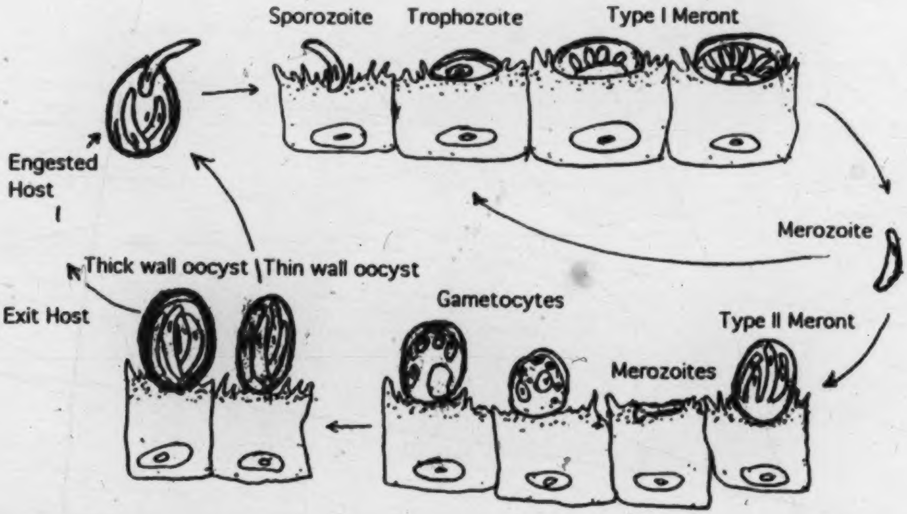
LITERATURE REVIEW

2.1 CRYPTOSPORIDIUM PARVUM

2.1.1 Biology

Cryptosporidium spp. was first observed by Ernest Edward Tizzy in 1907. Taxonomically, *Cryptosporidium* spp. are classified within Domain Eukarya, Kingdom Protozoa, Phylum Apicomplexa, Class Coccidea, Order Eucoccidiorida, and Family Cryptosporidiidae (Fayer 1997). The *Cryptosporidium* species have been found universally in fish, reptiles, birds and mammals, but only a few species including *C. muris* and *C. parvum* are infective to mammals (Fayer et al. 1990). *C. parvum* is the only specie that causes human diarrhea disease called cryptosporidiosis (Current and Bick 1989).

The life cycle of *C. parvum* has been well documented (Fayer and Ungar 1986; Current and Garcia 1991) (Figure 2-1). Mature *C. parvum* oocysts are shed in the feces of infected hosts, then through a number of routes including contaminated water or food the oocysts are ingested by other suitable hosts. In the gastrointestinal tract, the sporozoites excyst from the oocysts and invade the cellular epithelial cells of the intestine. The subsequent developmental stages are intracellular, surrounded by the membrane between parasite and host. The sporozoites differentiate into spherical trophozoites, then asexual multiplication occurs to form two types of meronts. Upon maturation, type I meronts develop into six to eight merozoites, and many of them invade new host cells and develop either into type I meronts, or type II meronts. A type II meronts differentiate into four merozoites, which invade new host cells and differentiate into either microgametocyte or macrogamont stage. The macrogamont fertilized by the microgametocytes becomes zygote, which eventially develop into thin wall or thick wall oocysts. The thin wall oocyst can auto-infect the same host, while the thick wall oocysts are released in the feces and can survive outside the host. The dormant stage of the parasite life cycle is important for transmission of infection to new hosts.


Sporozoite
Trophozoite
Type I Meront
Engested
Host
Merozoite
Thick wall oocyst
Thin wall oocyst
Exit Host
Gametocytes
Type II Meront
Merozoites

After Current and Garcia (1991).

Figure 2-1. Life cycle of *Cryptosporidium parvum*

The thick wall *C. parvum* oocysts are characterized by two distinctive oocysts wall layers and four sporozoites surrounding a large residuum containing amylopyctin granules (Current and Reese 1986). *C. parvum* oocysts can survive in water and soil for more than 12 weeks at –4 to 4°C (Olson et al. 1999). *C. parvum* oocysts are about 5 µm in diameter, and have been shown to be able to go through the conventional sand filter (Karanis et al. 1998). The parasites are very resistant to the pH or chemical exposures that are associated with the traditional water treatment processes (Robertson et al. 1992). Oocysts may lose their viability after 4 h desiccation at room temperature (Robertson et al. 1992). They are killed at 72.4°C within 1 min (Fayer 1994a).

2.1.2 Occurrence and Detection

Outbreaks of cryptosporidiosis have been frequently reported in North America, Scotland and United Kingdom and other countries (Hayes et al. 1989; Bell et al. 1993; Roefer et al. 1996; Solo and Neumeister 1996). In the Milwaukee outbreak in 1993, more than 400,000 people were exposed to *C. parvum* (MacKenzie et al. 1994; Fox and Lytle 1996). Cryptosporidiosis caused by person to person contact and contaminated food or recreational water has also been reported (Bell et al. 1993; Fayer 1994b; Lake and Hasell 1996).

The occurrence of *C. parvum* oocysts in water is ubiquitous. Even in water bodies not obviously affected by human activities, oocysts have been detected in more than 70% of the samples (Lisle and Rose 1995). Oocysts have also been found in sub-

Arctic area such as Yukon and Alaska (Roach et al. 1993; Pollen et al. 1996). A survey (Hancock et al. 1998) of the US groundwater showed that 5 to 50% of the groundwater was contaminated with the parasite.

During the last decade, a lot of work has been done in monitoring the oocysts in water, but the monitoring method are generally unreliable (Clancy et al. 1994; 1999). One of the difficulties in monitoring of the oocysts is a variable recovery rate. At present, the IFA (Immunofluorescence Assay)-Percoll-sucrose gradient method, which is referred to as the EPA method, has an average recovery rate of 74% (LeChevallier et al. 1990). Emerging technologies for oocyst detection include the flow cytometry with cooled couple devices (Campbell et al. 1993a), antibody-coated magnetic particles capture and ELISA (enzyme-linked immunosorbent assay) detection (Whitmore and Carrington 1992; Grabow et al. 1993). However, these methods do not assess parasite viability.

2.1.3 Oocyst Viability Assessment

2.1.3.1 Infectivity

In vivo infectivity bioassay is the standard reference method of *C. parvum* oocyst viability. The neonatal CD-1 mice model has been often used for infectivity evaluations. ID_{50}, termed inoculum that causes 50% of the mouse cohort to become infected, was initially estimated to be 79 oocysts per mouse for this model (Finch et al. 1994b). Further study analysis of infectivity data, however suggested that the ID_{50} of CD-1 mice was batch specific varying from 60 to 347 oocysts per mouse (Gyürék et al. 1999). Other strains of mice, such as BALB/c, have also been used for infectivity assay (Novak and Sterling 1991; Fayer et al. 1998) but no response models have been developed. From limited studies of human infectivity (DuPont et al. 1995), the ID_{50} of *C. parvum* to adult humans was estimated to be 132 oocysts for adult humans. After 1 year primary challenge, a dose of 500 oocysts per person in the re-challenge resulted in 3 out of 19 persons being infected, and the ID_{50} in the re-challenge was estimated to be 1,880 oocysts per person (Okhuysen et al. 1998; Chappell et al. 1999).

2.1.3.2 Excystation

In vitro excystation is an alternative method for evaluation of the viability of *C. parvum* oocysts. Usually excystation is conducted at 37°C and pH 7 (Fayer and Leek 1984), and in the presence of taurocholic acid (Woodmansee 1987). The maximum excystation generally occurs within 1 to 4 h of incubation (Campbell et al. 1992). The excystation rate of *C. parvum* oocysts may vary between isolates, and is sensitive to different excystation conditions and pre-incubation procedures. Excystation has been shown not to correlate with infectivity evaluation. Neumann et al. (2000a) showed that the intact oocysts after an excystation procedure, were highly infective to the CD-1 mice. Comparison of excystation and infectivity showed that the results from these two methods were not comparable (Finch et al. 1993a). Black et al. (1996) reported that both *in vitro* excystation and DAPI/PI (4 prime 6-diamidino-2-phenyl indole/propidium iodide) staining over-estimated the oocyst viability after chemical inactivation. It appears that excystation is not a very reliable procedure for estimation of parasite viability.

2.1.3.3 Vital Dyes

Different vital dyes have been tested for the detection of *C. parvum* oocyst viability. Fluorescent diacetate (FDA) and propidium iodide (PI) staining were first used for viability evaluation of *Giardia* spp. cysts (Schupp and Erlandsen 1987), but it was reported unreliable for *C. parvum* oocysts (Korich et al. 1990a). Another vital dye method, employing the fluorogenic vital DAPI and PI, was reported to be correlative to *in vitro* excystation (Campbell et al. 1992; 1993b). Recently, a new nucleic acid SYTO-9 vital dye method was developed for *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts (Taghi-Kilani et al. 1996; Neumann et al. 2000b) and was shown to be correlative to animal infectivity. The vital dye methods are based on the difference of oocysts metabolic potential between live and dead parasite and dependants on oocyst wall permeability.

2.2 WATER MICROORGANISM REDUCTION

2.2.1 Control of *C. parvum* in Water

Multi-barriers concept is employed for the design and operation of water treatment facilities for removal of chemical and biological contaminants. For control of pathogenic microorganisms, usually three strategic steps, named source control, physical removal and microbial reduction, are essential to water supply systems. Microbial reduction processes are the last barriers for protection of the consumer from waterborne pathogens, therefore, it is critical in water treatment.

Source control reduces the number of pathogenic microorganisms in the source water by watershed management. *C. parvum* that are potentially infective to humans are also present in numerous hosts such as domestic and wild animals. Studies showed that human activities such as farming and recreation near the watershed were related to the concentration of *C. parvum* oocysts in the surface water (Ong et al. 1996). Major watershed characteristics that affect *C. parvum* oocyst concentration in surface water include: level of human recreational use, intensity of dairy farming, size of wildlife population and domestic animals, and the presence of wastewater treatment plant discharge (Hansen and Ongerth 1991).

Physical removal of oocysts in water treatment facilities was achieved by multiple barriers including coagulation, flocculation, sedimentation, dissolved air flotation and filtration. The conventional clarification or the flotation plus filtration may reduce oocysts effectively. The pilot-scale study of dissolved air flotation (DAF) plus filtration showed that a 3.7 log-units removal could be achieved for the influent water containing 300 to 800 oocysts/L when the coagulation process was operated properly (Hall et al. 1995). Rapid sand filtration can effectively remove suspened particles in the size range of 0.1 to 50 μm. Up to 3 log-units of removal was achieved using different multimedia filters (Nieminski and Ongerth 1995; Ongerth and Pecoraro 1995). It was reported that slow sand filtration could achieve up to 4.5 log-units removal of *C. parvum* oocysts (Timms et al. 1995), whereas another study (Fogel et al. 1993) suggested a much lower removal efficiency of 48%. Shuler et al. (1991) reported that the diatomaceous earth filter could achieve 3 log-units of oocyst removal without using alum or cationic polymer. Some membrane filtration techniques, such as ultra-filtration and nano-filtration, theoretically can remove all the *C. parvum* oocysts (Jacangelo et al. 1991).

2.2.2 Microbial Reduction Chemicals

2.2.2.1 Chlorine Species

Chlorine is the most widely used microbial reduction chemical in water treatment. It is often used as an oxidizing agent for taste and odor control, color removal, oxidation of iron (II) and manganese(II), and ammonia removal (Snoeyink and Jenkins 1980; Bryant et al. 1992). Usually chlorine is applied as gas while it can also be applied as salt hypochlorous acid such as sodium hypochlorite. Normally, the chlorine gas reacts with water to form hypochlorous acid (HOCl).

$$Cl_2 + H_2O \leftrightarrows HCl + HOCl \qquad (2\text{-}1)$$

Addition of chlorine as a gas lowers the alkalinity of water due to the formation of strong acid (HCl). From the reaction we can also see that only half of the chlorine becomes active disinfecting agent (HOCl). At pH > 7, the hypochlorous acid begins to dissociate to form hypochlorite ion (OCl^-), which is less reactive than gas chlorine and hypochlorous acid (Andrade and Serrano 1993).

$$HOCl \leftrightarrows H^+ + OCl^- \qquad (2\text{-}2)$$

The term free available chlorine (FAC) refers to molecular chlorine (Cl_2) and its derivatives, hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). The distribution of free available chlorine, hypochlorous acid and hypochlorite ion depends on water pH and temperature. Hypochlorous acid is the major component at pH 6. At pH higher than 8, hypochlorite ion becomes the predominant chlorine species. At 20°C, HOCl constitutes 97.5, 79.3, and 27.7% of free chlorine at pH 6.0, 7.0, and 8.0, respectively (White 1992).

In the presence of ammonium in water, HOCl reacts with NH_4^+ through a stepwise reaction to form chloramines, which consist of three species: monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3) (Haas 1990).

$$HOCl + NH_4^+ = H_2O + NH_2Cl + H^+ \qquad (2\text{-}3)$$

$$HOCl + NH_2Cl = H_2O + NHCl_2 \qquad (2\text{-}4)$$

$$HOCl + NHCl_2 = H_2O + NCl_3 \qquad (2\text{-}5)$$

The species of chloramine formed depends on a number of factors including the chlorine to ammonium-nitrogen mass ratio, pH and temperature. For chlorine to ammonium (as nitrogen) mass ratios less than 5:1 at pH > 7, the predominant species formed is monochloramine (Snoeyink and Jenkins 1980). At lower pH values or higher chlorine to ammonium-nitrogen mass ratios, dichloramine may also be formed. The monochloramine is a relatively stable compound, which helps to maintain the chlorine residual concentration in the water distribution system. Less disinfection by-products, such as THMs, form when chloramine is used as an alternative for free chlorine (Norman et al. 1980; Ireland 1991).

The breakpoint chlorination method is often used in water disinfection (Bryant et al. 1992). In water with ammonium, the addition of chlorine results in the reaction of free chlorine and ammonium to form combined chlorine. when the Cl : N mass ratio reaches 5, further addition of chlorine results in the decrease of chlorine residual due to the formation of dichloramine. Theoretically, the break point is reached at the Cl : N mass ratio of 7.6. Stable free chlorine residual will exist when more chlorine is added after the breakpoint.

2.2.2.2 Ozone

Ozone is characterized by strong oxidizing properties. The oxidation-reduction potentials from ozone (O_3) to oxygen (O_2) is 2.07 V (Degrémont 1979).

$$O_{3(g)} + 2H^+ + 2e^- = O_2 + H_2O \qquad E_\emptyset=2.07 \qquad (2\text{-}6)$$

Ozone can react with constituents directly, and can also oxidize the constituents by the path of forming highly reactive chemical agents, including hydroxyl free radical ($OH\cdot$), ozonicle free radical anion O^{3-}, surperoxide free radical anion (O^{2-}), perhydroxyl free radical anion (HO_2^-) and hydrogen peroxide (H_2O_2) (Hoigné and Bader 1978; Bryant et al. 1992). The formation of these reactive agents depends on water characteristics, such as pH, carbonate, and organic compound concentration, which may significantly effect the outcomes of ozonation (Guittonneau et al. 1992; Beltran et al. 1993).

Ozone decomposes by two ways: one is direct decomposition and the other is cycle decomposition (Tomiyasu et al. 1985). Cycle reaction often contribute to the major portion of ozone decomposition (Bryant et al. 1992). The reaction begins with O_3 entering the cycle reaction and ends with the external product O_2. Three factors, named initiator, promoter and scavenger, affect this decomposition.

The initiators start the decomposition cycle and increase the level of hydroxyl radicals. A common initiator is hydroxide ion. Therefore, pH plays a key role in ozone decomposition. Ozone is very stable at pH 2 (Hoigné and Bader 1983). However, its decomposition becomes significant at pH>8, and at pH> 9 residual ozone is very difficult to maintain. Hydrogen peroxide and ultraviolet radiation are also initiators. Once the decomposition cycle begins, its reaction rate is controlled by the promoter and the scavenger. Ozone itself is a promoter and provides potential for continuing the cycle reaction. Other promoters, such as hydrogen peroxide, UV and formic acid, increase the concentration of radicals and promote the formation of superoxide (O_2^-), by which they accelerate the cycle reaction.

Scavengers slow down the ozone decomposition by stripping the hydroxyl radical from the circle reaction to form other products. Common scavengers presented in water are carbonate and bicarbonate. The presence of 1 mM carbonate scavenger can significantly slow the ozone decay rate (Staehelin and Hoigné 1982). Other common scavengers are bromide ion, acetic acid, phosphate ion and tert-butyl alcohol.

2.2.2.3 Chlorine Dioxide

Chlorine dioxide (ClO_2) is a strong oxidant. The standard oxidation-reduction potential for chlorine dioxide to chlorite ion (ClO_2^-) is 1.15 V (Degrémont 1979). The chlorine dioxide oxidization in water involves two steps. Firstly, chlorine dioxide gains one electron to form chlorite ion (ClO_2^-)

$$ClO_2 + e^- = ClO_2^- \qquad E_0 = 1.15\ V \tag{2-7}$$

Then chlorite ion gains four electrons to form chloride ion

$$ClO_2^- + H_2O + 4e^- = Cl^- + 4OH^- \tag{2-8}$$

Chlorite ion is less reactive to many constituents found in water, so the second step might not be completed in most water. Under the typical water treatment conditions, about 50 to 70 % of chlorine dioxide is converted to chlorite ion (Aieta et al. 1984).

Chlorine dioxide is relatively stable in neutral and mildly acidic solution. However, under alkaline conditions (pH > 8), a portion of the chlorine dioxide in the water solution could be degraded into chlorate ion (ClO_3^-) and chlorite ion (ClO_2^-) according to the following reaction

$$2ClO_2 + 2OH^- = H_2O + ClO_2^- + ClO_3^- \tag{2-9}$$

Chlorine dioxide is the active agent to *C. parvum* oocysts while chlorite ion and chlorate ion have little effect on oocyst viability (Liyanage et al. 1997a).

2.2.2.4 Combined Disinfectants

Combined disinfectants are two or more disinfectants applied simultaneously or sequentially. Typically, the strong oxidants serve as the primary disinfectant and long lasted chemicals serve as the secondary disinfectant. For example, monochloramine can be used after free chlorine primary disinfection, and chlorine species are used after ozone primary disinfection.

The initial purpose of adding secondary disinfectants was to control the bio-growth and provide a monitoring tool in the water distribution systems by maintaining the disinfectant residual. Nevertheless, addition of secondary disinfectant might have some extra benefit on the target microorganism inactivation. A synergistic effect might occur when the combined disinfectants were applied. Kott et al. (1980) studied the effects of ozone and chlorine applied individually, sequentially and in combination on the inactivation of *Salmonella typhimurium*, poliovirus type 1, T_2 coliphage and T_3 coliphage in secondary wastewater and artificially polluted tap water. They reported that simultaneous application of ozone and chlorine was superior to sequential application. In these experiments, the authors also reported chlorine to be more effective than ozone for inactivation of *S. typhimurium*, fecal coliform bacteria and *Streptococcus fecalis* whereas ozone was more effective for inactivation of poliovirus type 1, T_2 coliphage and T_3 coliphage. Unfortunately, the test apparatus, methods, and water quality were not sufficiently described in the paper to fully understand these results especially because ozone has been shown to be more effective against enteric bacteria than chlorine (Nieminski and Bradford 1991; Korol et al. 1995).

Katz et al. (1994) studied the effect of combined equal doses of chlorine dioxide and chlorine on the inactivation of indicator organisms contained in activated sludge. These authors reported an improved inactivation of total coliform, fecal coliform, fecal streptococci, and coliphages following combined application of 5 mg/L each for chlorine dioxide and chlorine. However, Katz et al. (1994) did not report the expected inactivation which would result from a single application of each disinfectant.

2.2.3 Factors in Microorganism Reduction

2.2.3.1 Temperature

Temperature increase accelerates the chemical reaction rate between disinfectants and microorganisms. The increase in temperature also causes a faster decomposition of the disinfectants. Farooq et al. (1978) studied bacteria inactivation using ozone at different temperatures and showed that the increase in reaction rate at higher temperatures was greater than the effect of decrease in ozone residual. Significant increase in inactivation of *Giardia muris* cysts by chlorine at high temperature was also reported by Labatiuk et al. (1992). In regulation of the Ct requirement for *Giardia* spp. inactivation by the US EPA, a factor of 2 was used for every 10°C decrease in water temperature within the range of 1 to 25°C (Malcolm Pirnie Inc. and HDR Engineering Inc. 1991).

2.2.3.2 pH

The effect of pH on microorganism reduction includes its effect on the properties of microorganism reduction chemicals and the susceptibility of microorganisms to chemical treatments.

The properties of disinfectants and their rate of decomposition may vary with water pH. Although there is no evidence that the inactivation capacity of ozone is sensitive to pH, ozone decomposes more rapidly at alkaline pH because OH^- is an initiator of ozone decomposition, resulting in less ozone being available for the reaction (Forni et al. 1982). Between pH 5 and 8, the proportion of hypochlorous acid decreases with water increasing pH. Lower pH is favorable for chlorination because hypochlorous acid is more effective than hypochlorite ion for microbial reduction processes. Chlorine dioxide is relatively stable in the pH range found in water treatment (pH 6 to 11), although its decomposition rate is higher at high pH. Hoff et al. (1981) reported a substantial increase in the microbial reduction capacity of chlorine dioxide at high pH.

The concentration of hydroxyl radical, an important intermediate product in ozonation, has a higher oxidation potential than ozone itself (for hydroxyl radical Eø = 2.76 V, for molecular ozone Eø = 2.07 V). At pH 10.5, 1 mol ozone may produce 0.55 mol high energy radicals (Hoigné and Bader 1976), which could significantly affect the outcome of ozonation. Hydrogen peroxide, UV and high OH^- are applied to ozone oxidation to take the advantage of this hydroxyl radical oxidation, termed advanced oxidation. Hydroxyl radical has been shown to be more effective than ozone for destruction of some recalcitrant taste and odor compounds (Wolfe et al. 1989a). However, since the half-life of the hydroxyl radical is very short and its oxidation reaction is not selective, its ability to inactivate pathogens is limited. Wolfe et al. (1989a; 1989b) reported that, at the same ozone Ct value at about pH 8, the inactivation of *E. coli* and *G. muris* by PEROZONE or ozone alone was comparable, which suggested that the hydroxyl radical concentration did not have significant inactivation effect on those microorganisms.

The susceptibility of microorganisms altered by pH varies among different microorganisms and disinfectants. Farooq et al. (1976; 1977) reported that pH only affected the ozone decay but not its disinfection capacities. From the results of hypochlorous acid inactivation of *C. parvum* (Gyürék et al. 1997), wall permeability of the oocyst was the same for pH 6 to 10. Nevertheless, Labatiuk et al. (1992) reported that pH was a significant factor in ozone disinfection of *G. muris*. Studies of the oocyst surface (Drozd and Schwartzbrod 1996; Ongerth and Pecoraro 1996) suggested that the surface charge of the oocysts changed at different water pH values, and the Zeta potential of the oocysts was reported to be -25 to -35 mV at neutral pH, with the zero isoelectric point at pH 2.5. However, no study has examined the effect of the change in surface electric charge as related to the oocyst susceptibility to chemical inactivation.

2.2.3.3 Organic or Inorganic Compounds

In addition to water pH and temperature, the organic and inorganic materials in the water may have direct or indirect effect on the outcomes of water pathogen inactivation. Some organic and inorganic compounds may consume chemical disinfectants, resulting in decrease of the concentration of disinfectants available for reacting with microbes. Ammonium was shown to react with chlorine, causing a change of free chlorine to combined chlorine. Theoretically, presence of carbonate and bicarbonate in the water may change the decomposition rate of ozone (Yurteri and Gurol 1988).

The organic or inorganic components in water may also change the properties of some microorganisms. Attachment of these organic or inorganic compounds to the microbes may protect them form chlorination (Herson et al. 1987). Oocysts present in natural water often aggregate and clump. The aggregation of pathogen may increase their resistance to chemical inactivation. Clark et al. (1994) reported that microorganisms exhibit increased resistance due to clumping, aggregation, particle association, or modification of antecedent growth conditions. Specifically, the *Vibrio cholerae* was highly resistance to chlorination under those conditions. Chen et al. (1985) showed that aggregation reduced the efficiency of chlorine dioxide inactivation of *Naegleria gruberi* cyst by 65% to 85%.

2.2.4 Disinfection By-Products (DBPs)

Chemical disinfectants are biocides, and at high doses, they may affect human health. US EPA (1998a) set the maximum residual disinfectant levels (MRDLs) as 4.0 mg/L for chlorine and chloramine (as gas Cl_2) and 0.8 mg/L for chlorine dioxide.

Chlorine is a strong oxidizing agent and will react with organic compounds such as fulvic and humic acids to produce chlorinated organics such as trihalomethanes (THMs) and haloacetic acids (HAAs). THMs and HAAs are potential carcinogens (White 1992; Bull et al. 1995). Maximum contaminant level of total THMs and HAAs regulated by US EPA (U.S. Environmental Protection Agency 1998a) were 0.080 mg/L and 0.060 mg/L, respectively. When compared to chlorine, chlorine dioxide disinfection produces considerably fewer by-products by reacting with organic compounds (Rav Acha 1984; 1985; Werdehoff and Singer 1987). However, the chlorate ion and chlorite ion that degrade from chlorine dioxide also have adverse effect on human health (Lykins et al. 1990). Bromate ion (BrO_3^-) is one of the principal by-products of ozonation in bromide ion-containing source waters (Bourgine et al. 1993; Ko et al. 1996; Orlandini et al. 1997; Siddiqui et al. 1998). US EPA (1998a) promulgated a maximum contaminant level (MCL) for bromate ion of 0.010 mg/L due to its adverse effect on human health (Bull 1992).

2.3 CHEMICAL INACTIVATION OF *C. PARVUM* OOCYSTS

2.3.1 Single Chemicals

2.3.1.1 Ozone

A number of research groups have investigated chemical inactivation of *C. parvum* oocysts at bench-scale (Peeters et al. 1989; 1990a; Korich et al. 1990b; Perrine et al. 1990; Parker et al. 1993; Ransome et al. 1993; Finch et al. 1994b; 1997; Oppenheimer et al. 1997). In general, they conluded that ozone was the most effective chemical for inactivation of *C. parvum* oocysts.

Finch et al. (1994b; 1997) used animal infectivity for viability assessment of *C. parvum* oocysts and developed a kinetic model for ozone inactivation. At 22°C, the non-linear Incomplete gamma Hom model (I.g.H.) was found adequate for the description of ozone inactivation between pH 6 and 8 (Gyürék et al. 1999). Another research group (Rennecker et al. 1997; 1999) used the *in vitro* excystation method for viability assessment and developed a linear model for ozone inactivation of *C. parvum* oocysts. They reported that the Ct requirement increased by three times for every 10°C decrease in temperature within the temperature range of 5 to 30°C. In contrast, results of our work using CD-1 mice infectivity to determine oocyst viability (Finch and Li 1999) showed that ozone efficacy was reduced about half for every 10°C decrease in temperature with the temperature range of 1 to 22°C. At low temperature, the required Ct values from our study were lower than those reported by Rennecker et al. (1999) for the same inactivation level.

Owens et al. (1994; 1995) studied ozone inactivation of *C. parvum* oocysts in filtered Ohio River water at pilot plant scale. While the difficulties in this type of study were significant, they were able to verify that the disinfection criteria that had been developed earlier using bench-scale approach (Finch et al. 1994b) were reasonable for ozone inactivation.

2.3.1.2 Chlorine Dioxide

The inactivation of *C. parvum* oocysts using chlorine dioxide has been investigated by several groups (Peeters et al. 1989; Korich et al. 1990b; Ransome et al. 1993; Finch et al. 1997). Peeters et al. (1989) used low concentrations of chlorine dioxide (0.43 and 0.31 mg/L) in inactivation of *C. parvum* oocysts. They reported that about 1 log-unit of inactivation was caused by 0.43 mg/L chlorine dioxide and contact time of 30 min (Ct value of 13 mg·min/L) at neutral pH and room temperature. Korich et al. (1990b) studied chlorine dioxide inactivation of *C. parvum* oocysts at pH 7 and 25°C. A crude estimate of inactivation was made from their data using a dose-response model for CD-1 mice reported by Finch et al. (1993b). These calculations gave 2 log-units inactivation of oocysts with 1.3 mg/L initial residual of chlorine dioxide and contact time of 45 min or a Ct product of 59 mg·min/L. Ruffell et al. (2000) used *in vitro* excystation to study chlorine dioxide inactivation of *C. parvum* at 4 to 30°C and pH 6 to 10. They reported a greater inactivation rate at pH 10, and the inactivation rate based on the linear model increased by an average factor of 3.4 for every 10°C temperature increase. From the preliminary studies in our laboratory on chlorine dioxide inactivation (Li and Finch 1998), every 10°C decrease in water temperature resulted in 50% reduction in chlorine dioxide inactivation efficacy.

2.3.1.3 Chlorine Species.

Earlier studies reported that chlorine solutions had little effect on oocyst viability

(Campbell et al. 1982; Sundermann et al. 1987; Smith et al. 1988; Ransome et al. 1993). These studies often used chlorine bleach concentrations in the 1,000's of mg/L and induced less than one log-unit of inactivation. An earlier AWWA Research Foundation project in our laboratory examined chlorine inactivation of *C. parvum* oocysts using free chlorine and monochloramine, singly and in combination (Finch et al. 1997; Gyürék et al. 1997). The results showed that up to 1 log-unit of inactivation was achieved by about 2,000 mg·min/L chlorine Ct at room temperature (22°C). Kinetic models for *C. parvum* oocyst inactivation at room temperature using free chlorine or monochloramine were reported by Gyürék et al. (1997). Clearly, the concentration and contact time of these chemicals that could cause significant inactivation were too high to be practical for the water industry. There are no data for chlorine or monochloramine inactivation at very low temperatures and kinetic models for different temperatures are not presently available.

2.3.2 Multiple Chemicals

2.3.2.1 Ozone and Chlorine

A series of experiments was conducted in our laboratory to investigate the synergistic effect in inactivation of *C. parvum* oocysts using ozone and other chemicals (Gyürék 1997; Liyanage et al. 1997b), and showed evident synergy of sequential treatment. From the results of a previous AWWA Research Foundation project studying the inactivation of *C. parvum* oocysts (Finch et al. 2000), ozone followed by free chlorine at pH 6 or ozone followed by monochloramine at pH 8 were the promising combinations to achieve significant synergy. After an ozone pre-treatment that normally induced 1.5 to 1.8 log-units inactivation of *C. parvum* oocysts, addition of 2 mg/L free chlorine for 860 min at pH 6 and 22°C resulted in a gross inactivation of 3.9 log-units. Further study on sequential inactivation of *C. parvum* in our laboratory using ozone followed by free chlorine (Li and Finch 1999) showed that the gross kill of sequential inactivation increased linearly with the free chlorine $C_{avg}t$ product, and that low temperature decreased the synergy. Driedger et al. (1999) studied ozone and monochloramine sequential inactivation of *C. parvum* oocysts in the buffer water and used *in vitro* excystation for viability assay. They reported that the monochloramine reaction rate increased 5 times after 0.26 log-units of ozone pre-treatment (ozone Ct=1.4 mg·min/L). No kinetic models for sequential inactivation of *C. parvum* oocysts were found in the literature.

2.3.2.2 Chlorine Dioxide and Chlorine

A study (Liyanage et al. 1997d) on chlorine dioxide-based sequential inactivation found that some synergistic effect existed for *C. parvum* oocyst inactivation using chlorine dioxide followed by free chlorine or monochloramine. Further study (Finch et al. 2000) confirmed that, given a chlorine dioxide primary kill of 0.5 to 1.4 log-units, significant synergy could be achieved using free chlorine or monochloramine Ct at 22°C. However, at 5°C, the synergistic effect of chlorine dioxide followed by chlorine species was less evident. Corona-Vasquez (1999) studied *C. parvum* inactivation using *in vitro* excystation for viability assessment, and reported synergy using chlorine dioxide followed by free chlorine or monochloramine.

From the literature, ozone and chlorine dioxide have been proven to be the most effective single chemicals for inactivation of *C. parvum* oocysts. However, data at low temperature and different pH values were limited, and there are no models to fully describe the microorganism reduction kinetics at a variety of temperature and pH conditions. The studies on sequential inactivation of *C. parvum* oocysts were also limited to room temperature, and effects of different factors including level of pre-treatment, Ct product of the secondary treatment and temperature have not been fully examined. Therefore, a thorough study on *Cryptosporidium* spp. inactivation using these single or combined chemicals is necessary for design and operation of these chemical disinfection processes.

CHAPTER 3

MATERIALS AND METHODS

3.1 PARASITOLOGY METHODS

3.1.1 Biosafety

Cryptosporidium parvum is a pathogen and was handled followed the laboratory biosafety level criteria (level 2) regulated by Centers of Disease Control and Prevention (CDC), U.S.A. Gloves must be worn when working with biohazard materials. The isolation of the *C. parvum* oocysts was conducted in a clean air laminar flow hood (Canadian Cabinets He4-97-T). Biohazardous wastes were stored in biohazard container and autoclaved at the same day of the experiment.

3.1.2 Production of *C. parvum* Oocysts

The strain of *C. parvum* oocysts used in this study was the "Iowa" strain originally isolated by Dr. Harley Moon (National Animal Disease Center, Ames, Iowa). This Iowa strain of *C. parvum* was produced in male neonatal Holstein calves (*Bos tauris*) using a modified method described by Musial et al. (1987). Calves, age 2 to 4 days were infected with 2×10^7 *C. parvum* oocysts and maintained on a diet of one part milk replacer and one part electrolyte solution during fecal collection (oocyst batch # 1 to

6 and 9, 10, 11). For other experiments (oocyst batch # 7, 8, 12 to 19), the infected dose was up to 1×10^8 oocysts per animal and the calves were fed electrolyte solution only during the period of feces collection to reduce the fat content in the feces.

Feces containing *C. parvum* oocysts were collected from day 4 to day 10 post infection (PI). The feces were collected in tap water and sequentially passed through 10, 20, 60, 100, 200 and 400 mesh sieves (Fisher Scientific Inc.) by agitating and washing the sieves with 0.01% Tween 20 (v/v). Concentration of the particulates from the sieved feces was done by centrifugation at 1,100 × g for 10 min.

The purification of oocysts from pelleted fecal material was done using cesium chloride (CsCl) gradient ultracentrifugation. A CsCl gradient was prepared in a 40 mL Beckman polyallomer ultracentrifuge tube. The placement consisted of a bottom layer (7 mL of 1.4 g/mL CsCl), middle layer (11 mL of 1.1 g/mL CsCl) and a top layer (9 mL of 1.05 g/mL CsCl). Approximately 5 mL of the parasite material was gently layered on top of the CsCl gradient and centrifuged at 16,000 × g for 60 min using a swinging-bucket rotor (SW-28) at the slow brake setting (Beckman L7-55 ultracentrifuge). After centrifugation, the band containing the purified oocysts was removed using a pipette and placed in 50 mL polypropylene tubes. The tubes were filled with deionized water and the oocysts washed twice by centrifugation at 14,500 × g for 10 min using a fixed-angle SS-34 rotor for a high-speed centrifuge (Sorval, RC5-B centrifuge). After the final washing step, the oocysts were suspended in deionized water containing 100 U of penicillin, 100 µg/mL of streptomycin, 100 µg/mL of gentamicin and 0.01% Tween 20 at 4°C prior to use in experiments.

In cases where low numbers of parasites were found in fecal samples, oocysts were concentrated in fecal material using sucrose flotation before CsCl gradient centrifugation. A 50 mL conical centrifuge tube was filled with 30 mL of sucrose solution (1,320 g/L of water) onto which 500 µL of emulsified feces was layered. The tubes were mixed by inversion and centrifuged at 800 × g for 10 min at 4°C. The oocysts found at the feces-sucrose interface were removed using a pipette and diluted five times using deionized water containing 0.01% Tween-20. The oocysts were washed three times in deionized water containing 0.01% Tween-20 at 28,000 × g for 20 min at 4°C. This enriched fecal suspension containing the oocysts was then layered onto CsCl gradient as described above.

Oocysts isolated by this procedure were free of debris and bacteria. Oocysts were used within 90 days of isolation in all experiments. The oocyst concentration in the suspension was determined by quadruplicate counts using a hemocytometer.

3.1.3 Animal Infectivity Assay

A neonatal CD-1 mouse model was used to evaluate infectivity of *C. parvum* oocysts (Finch et al. 1993b; Gyürék et al. 1999; Neumann et al. 2000a). Breeding pairs of CD-1 mice were obtained from Charles River Breeding Laboratories (St. Constant, Quebec, Canada). The animals were given food and water *ad libitum* and were housed in cages with covers fitted with a 0.22 μm filter in a specific pathogen-free (P-2 level) animal facility.

Oocyst doses were prepared from the stock suspension by serial dilution to obtain the required number of parasites. The actual dose given to individual mice was confirmed by quadruplicate hemocytometer counts of the final parasite suspensions. Five-day-old neonatal mice were inoculated intragastrically with 50 μL of deionized water containing a known number of oocysts. Intragastric inoculutions were done using a 24-gauge ball-point feeding needle (Popper and Sons). One hour prior to infection, the neonatal mice were taken away from mothers to ensure that their stomachs were empty and ready to receive the intragastric inoculum of *C. parvum*. In addition, neonates from multiple litters were pooled and randomly selected for infection, in order to minimize variability introduced by inherent resistance or susceptibility of neonatal littermates to infection with *C. parvum*.

C. parvum infections in mice were assessed by staining mouse intestinal homogenates with fluorescein-labelled anti-*C. parvum* monoclonal antibody (Immucell, Portland, ME, USA) and using flow cytometry to detect the presence of fluorescent oocysts (FASCalibur, Becton Dickinson, Franklin Lakes, NJ). Mice were killed by cervical dislocation and the lower half of the small intestine, cecum, and colon removed and placed in 10 mL of deionized water. The intestines were homogenized for 45 to 60 s in a Sorvall Omni-Mixer and washed once with deionzed water containing 0.01% Tween

20 at 2,000 × g for 15 min. The supernatant was discarded and the cell pellet disrupted by vigorous mixing. Twenty microliters of the viscous pellet was pipetted into a 35 µm sieve fitted onto a 6 mL polystyrene tube (Becton Dickinson), and the sieve flushed with 400 µL of 0.1% BSA (fraction V, Boehringer Mannheim) in phosphate buffer solution (PBS). The strained suspension was incubated for 15 min at room temperature in order to block non-specific adsorption of monoclonal antibodies to intestinal contents. One hundred microlitres of a 1:400 dilution of a fluorescein-labelled anti-*C. parvum* monoclonal antibody was added to each of the tubes and incubated at 37°C for 30 min (final antibody dilution = 1:200).

Detection of *C. parvum* oocysts was done using a FASCalibur flow cytometer programmed under the following settings: (1) forward light scatter photodiode setting=00 and amp gain=4.00, (2) side scatter photomultiplier setting=402, and (3) FL1 photomultiplier setting=470. Fifty thousand events were collected for each sample. A stock oocyst suspension was used to define a region based on size (*i.e.* forward light scatter) and internal complexity (*i.e.* side scatter) of *C. parvum* oocysts. This defined region was subsequently used to discriminate potential oocysts in mouse intestinal homogenate. An additional criterion (*i.e.* gate) within this region was defined based on the fluorescent staining intensity (*i.e.* FL1) of particles within this region. Thus, oocysts were discriminated from other intestinal particulate material having the same size and internal complexity based on the acquisition of specific anti-*C. parvum* fluorescence labelled mAbs on their surface.

The estimated infectious dose was calculated from a logistic dose-response model calibrated for each individual batch of oocysts as described earlier (Finch et al. 1993b; 1997). The form of the dose-response model is

$$\pi = \ln\frac{P}{1-P} = \beta_o + \beta_1 \log(X) \qquad (3\text{-}1)$$

where P = the proportion of the cohort infected for a given inoculum X; and β_o and β_1 = the logistic response model parameters. The inactivation ratio was estimated from

$$I_r = -\log\frac{N}{N_o} = -\log\left(\frac{n}{n_o}\right) \qquad (3\text{-}2)$$

where I_r = logistic inactivation level (log-units); N = number of surviving oocysts after treatment, N_o = number of live oocysts before treatment; n = the estimated infectious dose per animal after disinfection from the dose-response model; and n_0 = the infectious number of oocysts given to each animal as estimated by the dose-response model in the control.

3.2 OXIDANTS AND APPARATUS

3.2.1 Chlorine

Free chlorine stock solution was prepared daily as needed using sodium hypochlorite solution (6% available chlorine, BDH Inc., Poole, England) with oxidant demand-free water to give a concentration of about 300 mg/L. Chlorine concentration was determined by forward amperometric titration or by the DPD procedure (Greenberg et al. 1992). The stock chlorine solution was stored in dark refrigerated conditions during the day of the experiment. Chlorine residual in the reactor after treatment was neutralized using sodium sulfite.

3.2.2 Monochloramine

A stock ammonium chloride (AnalaR grade, BDH Inc., Poole, England) solution (1,000 mg/L) was prepared as the ammonium source for preparing monochloramine. A daily working solution of monochloramine was prepared by mixing equal volumes of sodium hypochlorite and ammonium chloride solutions at a 3:1 (chlorine : nitrogen) mass ratio, yielding a 150 ± 10 mg/L (as chlorine) solution. Each solution was prepared in 0.05 M phosphate buffer (pH 8). This yielded approximately a 0.6:1 molar ratio (chlorine : nitrogen).

The combined solutions were stirred for 15 min, and the resultant solution was checked for free and monochloramine using forward amperometric titration or by the DPD procedure (Greenberg et al. 1992). The solution was used if no detectable species other than monochloramine were found. Monochloramine residual in the reactor was neutralized using sodium sulfite.

3.2.3 Ozone

Ozone gas was generated using a water-cooled corona discharge generator (Model

T-816, Welsbach Ozone Systems Corporation, Sunnyvale, CA) from extra dry oxygen feed gas. Oxygen carrier gas containing approximately 5% ozone was bubbled for a minimum of 20 min at 20°C through 400 mL of deionized water in a 500 mL gas absorption flask. Ozone concentration in the stock solution was approximately 20 mg/L. When necessary, the stock concentration was increased to about 40 mg/L by lowering the temperature of the solution to 4°C. The ozone solution was used within 3 min of removing it from the gas stream. Ozone residual in the reactor was neutralized using sodium formate.

Ozone residuals were determined by UV absorbance at 260 nm when interference from absorbing substances was not significant. A molar absorptivity of 3,300 l/(M·cm) was used (Hart et al. 1983).

3.2.4 Chlorine Dioxide

Chlorine dioxide was generated by a chlorine dioxide generator (CDG Technology, Inc., New York, NY) following the procedures described elsewhere (Liyanage et al. 1997a). Briefly, a chlorine and nitrogen mixture gas with 4% chlorine was passed through a packed bed of sodium chlorite pellets where all of the chlorine was converted to chlorine dioxide gas. Product gas was captured in a 500 mL gas absorption flask containing 400 mL Elga ultra-pure water (Fisher Scientific Inc, Pittsburg, PA). Concentration of the captured chlorine dioxide stock solution ranged from 2,000 to 5,000 mg/L.

Concentration of chlorine dioxide and free chlorine in the captured stock solution were determined by the amperometric titration (Aieta et al. 1984). As a quality control, concentration of chlorite ion and chlorate ion in the captured chlorine dioxide stock solution were determined by ion chromatography (INOPAC AS9-SC Analytical Column, DIONEX Corp. Sunnyvale, CA) following the method describe by Pfaff (1993). For the chlorine dioxide stock solution that contained extra high concentration of impurities (total amount of free chlorine, chlorate ion or chlorite ion exceeding 5% of the chlorine dioxide in the stock solution), further purification was done using nitrogen aeration and re-capture in the reactor.

The captured chlorine dioxide was stored at 4°C in a 50 mL dark brown airtight bottle. Usually the chlorine dioxide stock solution was used within 2 months. Fresh chlorine dioxide working stock solution (about 100 mg/L) was prepared daily by diluting the captured stock solution into the oxidant demand-free water. Chlorine dioxide concentration in the working stock solution or in the treatment reactor was determined spectrometry at 360 nm using a molar absorptivity of 1,250 1/(M·cm) (Gordon et al. 1992). Chlorine dioxide residual after treatment was neutralized using sodium sulfite or sodium thiosulfate.

3.2.5 Oxidant Demand-Free Glassware

All glassware was initially cleaned using a detergent specifically designed for laboratory glassware and washed in deionized distilled water with an 20% phosphate acid rinse followed by two more high temperature rinses with deionized water. After the initial cleaning, all glassware was rinsed three times with deionized water including one more phosphate acid rinse. From then on, all glassware was cleaned using hot deionized water.

The disinfection reactor, stir bar, pipette tips and other glassware that may contact the test solution were made oxidant demand-free to reduce the oxidant demand. Glassware was made chlorine dioxide or chlorine demand-free using standard methods by exposing glassware to water containing at least 10 mg/L chlorine for 3 h or more before use and rinsing with chlorine demand-free water (Greenberg et al. 1992). It was found during the course of the experiments, that ozone demand-free water and utensils were also chlorine dioxide and chlorine demand-free. Therefore, laboratory procedures were streamlined to follow the ozone demand-free protocols and the prepared water and utensils are called "oxidant demand-free". Openings in the glassware were covered with fresh aluminum foil to prevent dust from entering the glassware.

3.2.6 Reactor Vessels

The reactor vessels used were oxidant demand-free, 250 or 500 mL Erlenmeyer flasks. The Erlenmeyer flask reactors were stirred using a Teflon-coated magnetic stir bar. The agitation speed was sufficient for rapid, complete mixing without creating a vortex. A similar reactor configuration has been used previously (Finch et al. 1994b).

When working with ozone, chlorine dioxide, free chlorine and monochloramine, reactor vessels were covered with aluminum foil to minimize volatilization and photodecomposition of disinfectants. A diode-array spectrophotometer (Hewlett-Packard Model 8452A) with a 10 mm light path and 35 μL flow through cell was operated in a closed loop to continuously monitor ozone and chlorine dioxide residual as described elsewhere (Finch et al. 1992; 1994b).

3.2.7 Temperature Control

Temperature in the reactor was controlled by an orbital shaking water bath with microprocessor control (Model 3545, LAB-LINE INSTUMENTS Inc., Illinois) that maintained a stable temperature within ± 0.1°C. The water bath was chilled using an off-line refrigerator when low temperature was needed for the experiment. The reactor was submerged in the water bath and used a submersible magnetic stirrer (VWB Scientific, Model 230) for mixing.

3.3 DETERMINATION OF OXIDANTS CONCENTRATIONS

3.3.1 Ozone

The concentration of ozone aqueous stock solution was measured by ultraviolet spectrophotometry (10 mm cuvette, Ultraspec 2000, UV/Visible spectrophotometer, Pharmacia Biotech Ltd., Cambridge, England) at 260 nm with the molar absorptivity of 3,300 1/(M·cm). Hart et al. (1983) indicated that the molar absorption efficient of ozone at pure water is 3292 ± 70 1/(M·cm). Due to the quick decay in ozone stock solution (half-life about 20 min for aqueous ozone at neutral pH in pure water and room temperature), the ozone stock solution was freshly prepared before each experiment. Before adding ozone stock solution to the reactor, aeration by the ozone product gas was stopped and the ozone stock solution was allowed to stand for 2 min to let the ozone gas escape from the water. Three measurements of ozone concentration in the stock solution were taken before applying it to the reactor. Three more measurements were taken right after applying ozone. The mean of these six measurements was considered the concentration of ozone stock solution in calculation of the applied dose.

The real time ozone concentration of in the reactor was also monitored by the ultraviolet spectrophotometry (HP 8452A Diode Array spectrophotometer, Hewlett-Packard Company) at 260 nm. The spectrophotometer sample pump continuously sampled the reactor contents using a closed loop at a flow rate of 8 mL/min. The cuvette holder of HP 8452A spectrophotometer was cooled by the circulating cooling water to prevent the fog forming on the cuvette surface at low temperatures.

3.3.2 Chlorine Dioxide

The concentrations of chlorine dioxide and free chlorine in the captured stock solution were determined by the amperometric titration (Aieta et al. 1984). Fresh chlorine dioxide working stock solution was prepared daily by diluting the captured stock solution into oxidant demand-free water. Concentration of the chlorine dioxide working stock solution was determined spectrophotometry (Ultraspec 2000, UV/Visible spectrophotometer, Pharmacia Biotech Ltd., Cambridge, England) at 360 nm with the molar absorptivity of 1,250 1/(M·cm) (Gordon et al. 1992). A cuvette with a light of path of 50 mm was used in the measurement. Usually, the mean of triplicate measurements was taken as the chlorine dioxide stock concentration. Real time chlorine dioxide concentration in the reactor was continuously monitored by spectrophotometry (HP 8452A Diode Array spectrophotometer, Hewlett-Packard Company) at 360 nm using a molar absorptivity of 1,250 1/(M·cm) (Gordon et al. 1992).

3.3.3 Chlorine and Chloramine

The concentration of chlorine and monochloramine stock solutions were determined by the DPD colorimetric procedure (Greenberg et al. 1992). Total chlorine and free chlorine powder pillows (HACH Company, Coveland, CO) for 25 mL sample volume were used in the measurement. A standard curve was prepared monthly using potassium permanganate solution prepared by dissolving 891 mg $KMnO_4$ (BDH AnalaR reagent grade, dried at 105°C for 1 h) into 1,000 mL oxidant demand-free water. This 1,000 mg/L stock soltion was diluted ten-fold to100 mg/L with oxidant demand-free water in a volumetric flask. One milliliter of this solution was diluted to 100 mL with oxidant demand-free water, to produce a chlorine equivalent of 1.00 mg/L in the DPD reaction. A serial dilution of $KMnO_4$ standards was prepared to cover the chlorine equivalent range of 0.05 to 4 mg/L. A DPD powder pillow was added to each $KMnO_4$ standard solution, then its absorbance at 515 nm (Ultraspec 2000, UV/Visible spectrophotometer, Pharmacia Biotech Ltd., Cambridge, England) was measured immediately. A standard curve was developed based on response of the absorbance *versus* equivalent free chlorine concentration.

The concentrations of free chlorine and monochloramine were measured using the free chlorine and total chlorine DPD powder pillows, respectively. The free chlorine or monochloramine was usually within the range of the standard curves. If not, the samples were diluted before re-measurement. Twenty-five mililitres of sample was put into a 50 mL oxidant demand-free Fisher brand borosilicate glass vial, and a package of DPD powder was added to the mixture. The absorbance was measured at 515 nm. In the disinfection experiment, usually 5 mL of sample was taken and diluted 5 folds for measurement of free chlorine and monochloramine concentration in the reactor.

3.4 WATER TYPE

Due to the nature of the experiments, it was desirable to isolate different sources of experimental error. Because the data from this study will be used widely, it was desirable to have a uniform, reproducible water quality. For this reason, deionized water was obtained from an Elga® system (ELGASTAT MAXIMA-HPLC, Elga Ltd. England) operated at a resistivity of at least 18 MΩ/cm. The standard 0.05 M phosphate buffer was prepared using potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate (BDH Inc. AnalaR Grade, England) adjusted to the desired pH of the experiments with a strong acid (0.1 M hydrochloric acid) or a strong base (0.1 M sodium hydroxide). The resulting solution was made oxidant demand-free by bubbling ozone through the solution for 40 min, followed by boiling for 20 min and cooling in a clean air hood. The pH of the phosphate buffer was measured (Accumet Model 25 pH/ion meter, Fisher Scientific) when it was cooled down to room temperature. This procedure was repeated until the measured pH was ±0.1 of the target pH value. The resulting solution was found to be ozone, chlorine dioxide, chlorine and monochloramine demand-free. The buffered solution was found to resist pH drift adequately under all oxidant dosing conditions. The oxidant demand-free water and buffer solution were stored in 4 L brown reagent bottles at room temperature and were used within 3 months.

3.5 MICROORGANISM REDUCTION PROCEDURE

Experiments were conducted in oxidant demand-free water using batch reactors containing between 1.0×10^6 and 1.0×10^8 *C. parvum* oocysts. The reactors were wrapped in aluminum foil and covered with a foil lip to minimize the volatilization and photodecomposition of the oxidants, and mixed by an oxidant demand-free Telfon-coated magnetic stir bar. The reactors, test water and oocysts were brought to the desired temperature slowly using the temperature controlled water bath. The oocysts were acclimated to the water bath for at least 60 min. Vigorous agitation to completely mix the oocysts was essential before adding the oxidants.

A control reactor was run side by side with the experimental series conducted for each trial day. The control was processed exactly the same as the experimental trial except that no oxidants were added. A third reactor was used to determine the disappearance of oxidants when needed. Occasionally, negative controls were performed to determine the behavior of the C. *parvum* oocysts in the test water.

3.5.1 Single Oxidants

Experimental protocols for treatment followed a modified procedure described elsewhere (Finch et al. 1994b). About 1.0×10^6 to 1.0×10^8 oocysts were used for each experimental trial. The number of oocysts used depended on number of oocysts required for the infectivity assessment, which was related to the level of kill and number of mice used for that trial. Stock oocysts purified from the calf were washed and concentrated before being suspended in the laboratory water. The exact number of oocyst was determined using a hemocytometer.

Typically, the oocysts were suspended in a 200 mL of oxidant demand-free 0.05 M phosphate buffer solution in a 250 mL Erlenmeyer flask. Oxidant was applied to the reactor by adding a measured volume of oxidant working stock solution using a calibrated pipette. The residual disinfectant concentration in the reactor was monitored continuously by ultraviolet spectrophotometry (HP 8452A Diode Array Spectrophotometer, Hewlett-Packard Company). At the end of the contact time, the residual disinfectant was neutralized by quenching reagents (1 M sodium formate for ozone, 0.05 M sodium sulfite or sodium thiosulfate for chlorine dioxide and 0.05 M sodium sulfite for chlorine species). Oocyst samples after treatment were stored in the 250 mL or 50 mL centrifuge tubes at 4°C and infectivity assessment was done within 12 h after disinfection procedure.

3.5.2 Sequential Oxidants

Serial sampling of oocysts from one reactor was done in the sequential experiments. The procedures for preparing test water and oocysts were similar to that used for a single disinfectant. Typically, 4×10^7 to 5×10^7 oocysts was used in a 250 mL Erlenmeyer flask with 200 mL buffer solution. About 20 mL of the sample was taken as a control before adding ozone stock solution. The real time ozone concentration in the reactor was monitored by HP 8452A diode array spectrophotometer. At the end of primary contact time, the residual of the primary disinfectant was removed by adding a sodium formate. The second sample (40 mL) was taken from the reactor representing the ozone primary kill. Immediately, the secondary disinfectant was applied by adding measured volume of free chlorine or monochloramine stock solution. The concentration of free chlorine or monochloramine residual in the reactor was measured by the DPD procedures (Greenberg et al. 1992). One or two more samples were taken from the reactor to measure gross kill at different secondary Ct conditions. The residual free chlorine or monochloramine were neutralized by 0.1 N sodium sulfite.

The kill by the secondary treatment alone was measured separately following the procedures used for single treatment. A kinetic model was developed for free chlorine or monochloramine disinfection, and the kills by free chlorine or monochloramine used singly were predicted by the kinetic models based on the concentration and contact time. The synergy of the sequential disinfection was calculated as

$$\text{Synergy} = I_r - (I_{r1} + I_{r2}) \tag{3-3}$$

where I_r = gross logistic inactivation of sequential disinfection measured in the experiment (log-units); I_{r1} = logistic inactivation by ozone primary inactivation measured experimentally (log-units); and I_{r2} = logistic inactivation by free chlorine or monochloramine alone, under the same concentration and contact time as they were used in the secondary treatment (log-units).

Under conditions that were examined, the kills by free chlorine or monochloramine alone were very low (less than one log-unit of inactivation at room temperature and almost no effect at low temperature). In that case, the model predicted kill based on the robust kinetic model instead of a single observation was used in the calculation to avoid high variance being introduced to the result.

3.6 KINETIC MODELING

3.6.1 Chick-Watson Model and I.g.H. Model

The generalized inactivation rate law with the parameters of the number of organisms, chemical concentrations, contact time and the first order disappearance of the chemicals is expressed as

$$\frac{dN}{dt} = -kmN^{x'}C_o^n e^{-k'nt} t^{m-1} \quad (3\text{-}4)$$

where dN/dt = rate of microorganism reduction; k = inactivation rate constant found experimentlly; N = number of survivors at the contact time t (min); C_o = the initial concentration of the chemical (mg/L); k' = the rate constant of the first order disinfectant decay (1/min); and m, n, x' = empirical constants. Assuming x=1, m=1 and integrating the equation yields the Chick-Watson model (Gyürék and Finch 1998)

$$\log\frac{N}{N_o} = -\frac{k}{k'n}(C_o^n - C_f^n) \quad (3\text{-}5)$$

where C_f = chemical residual at the end of contact time (mg/L). The widely used Ct concept simplifies the model by assuming n = 1. Further simplification of the model can be made by assuming constant chemical residual. In the case where the chemical loss or decay rate is low, the average of the initial and final residual is used to represent the chemical concentration (C_{avg}t model)

$$\log\frac{N}{N_o} = -kC_{avg}t \quad (3\text{-}6)$$

where $C_{avg} = (C_o+C_f)/2$ (mg/L).

In the case where n and m are not unity, integration of the equation (3-4) by assuming x=1 gives a robust Incomplete gamma Hom (I.g.H.) model, which can be used to describe the shoulder effect and tail-off effect of the survival curves (Haas and Joffe 1994).

$$\log\frac{N}{N_o} = -\frac{mkC_o^n}{(nk')^m}\cdot\gamma(m, nk't)\ ,\ m > 0, nk't \geq 0 \qquad (3\text{-}7)$$

$$\gamma(\alpha, x) = \int_0^x e^{-x} z^{\alpha-1} dz, \alpha > 0, x \geq 0$$

The incomplete gamma function $\gamma(m, nk't)$ can be expressed as GAMMADIS (nk't, m, 1, true) × EXP(GAMMALN(m)) in the MS EXCEL 97/98. Other software packages such as Mathcad 6 plus and Maple also have the required gamma functions.

3.6.2 Temperature Corrected Models

An empirical model that describes the dependence of the rate constant on temperature is the van't Hoff-Arrhenius relationship (Tchobanoglous and Burton 1991)

$$k = A \cdot e^{-\frac{E_a}{RT}} \qquad (3\text{-}8)$$

where A = the frequency factor; E_a = the activation energy (J/mol); R = the universal gas constant (8.31 J/(mol·K)); and T = the temperature (Kelvin). The activation energy of the reaction can be estimated by the reaction rate constants at two different temperatures (T_1 an T_2) using the following equation

$$E_a = \ln(\frac{k_1}{k_2})\cdot\frac{R \cdot T_1 \cdot T_2}{T_1 - T_2} \qquad (3\text{-}9)$$

where k_1 = the reaction rate constant at T_1; and k_2 = the reaction rate constant at T_2.

Solving the basic relationship for a narrow temperature range allows the term $\theta = e^{\frac{E_a}{R\,T_1\cdot T_2}}$ to be set as a constant, yielding

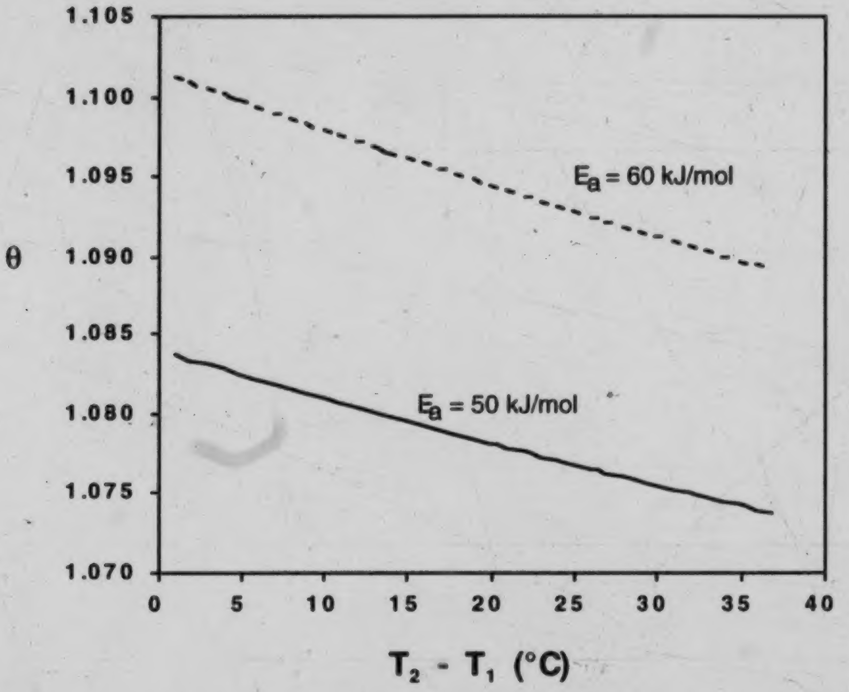


Figure 3-1. Temperature coefficient as a function of the range of temperature used for its estimation ($T_1 = 0°C$)

$$\frac{k_T}{k_{22}} = \theta^{T-22} \tag{3-10}$$

where k_{22} = the rate constant at 22°C; k_T = the rate constant at T (°C); and θ = the temperature coefficient which refers to the effect of temperature per 1°C.

Figure 3-1 illustrates the dependence of temperature coefficient, θ, on the temperature range, T_2 and T_1, used for the temperature coefficient estimation. Assuming the activation energy of 50 kJ/mol, the temperature coefficient was estimated as θ =

1.084 if $T_1 = 0$ and $T_2 = 1°C$, while $\theta = 1.078$ if $T_1 = 0$ and $T_2 = 20°C$. Therefore, for a narrow temperature of 20°C, θ can be assumed as a pseudo constant. Combining Equations 3-5 and 3-10, the temperature correction Chick-Watson model is expressed as

$$\log\frac{N}{N_o} = -\frac{k_{22}\theta^{T-22}}{k'\,n}(C_o^n - C_f^n) \tag{3-11}$$

Similarly, the temperature corrected $C_{avg}t$ model is

$$\log\frac{N}{N_o} = -k_{22}\theta^{T-22}C_{avg}t \tag{3-12}$$

From Equation 3-7 and 3-10, the four-parameter I.g.H. model (k_{22}, θ, m, and n) with temperature correction is

$$\log\frac{N}{N_o} = -\frac{mk_{22}\theta^{T-22}C_o^n}{(nk')^m}\cdot\gamma(m, nk'\,t)\ ,\ m > 0, nk'\,t \geq 0 \tag{3-13}$$

3.6.3 Empirical Model for Sequential Inactivation

In the sequential inactivation, the gross kill, I_r, is the sum of the primary kill I_{r1} and the secondary kill I_{r2}

$$I_r = I_{r1} + I'_{r2} \tag{3-14}$$

where I_{r1} = logistic inactivation induced by the primary treatment; and I'_{r2} = logistic inactivation caused by the secondary treatment. Assuming the inactivation caused by the secondary treatment follows the linear Chick-Watson model (Equation 3-12), the gross kill caused by the sequential inactivation could be expressed as

$$I_r = I_{r1} + k_{22}\theta^{T-22}C_{avg}t \tag{3-15}$$

where $C_{avg}t$ refers to the secondary treatment conditions; k_{22} and θ = the parameters specified for the combination of chemicals and levels of primary treatment. They can be evaluated experimentally.

3.7 EXPERIMENTAL DESIGN

3.7.1 Single Treatment

The concentration and time conditions used for each combination of experiments were generally based on the values achievable in practice, while maintaining an inactivation within the limits of detection. Data at two temperatures (1 and 22°C) were collected to study the effect of temperature, and some data were collected at 5, 13 and 37°C to study fitness of the model temperature correction. For ozone related inactivation, pH values were selected between 6 and 8, which is the common pH range found in water treatment. Due to the very high ozone decay at higher pH values, application of ozone at a pH higher than 8 might not be economical. Chlorine dioxide is relatively stable over a wide pH range. In some water treatment systems, the water pH values can reach up to pH 10 when a chemical softening process is applied, so the pH range for the chlorine dioxide experiment was between pH 6 to 11.

Each concentration and contact time combination was tested at least twice as an individual trial for the noted pH and temperature. Selection of doses and contact times for a given temperature and pH was based on the experimental design given by Box and

Lucas (1959). By randomizing trials, independent observations were obtained and the problems of serial correlation of the data were eliminated, Because eliminating serial correlation was necessary for statistically analyzing the data (Box et al. 1978; Draper and Smith 1981).

3.7.2 Sequential Treatment

For ozone-based sequential treatment, the factors investigated included the level of ozone primary kill, the Ct product of secondary treatment, and the water temperature.

In the ozone pre-treatment, the inactivation levels between 0.4 to 0.7 log-units was targeted for the low level of pre-treatment and between 1.4 and 1.7 log-units was targeted for the high level of pre-treatment. Chlorine or monochloramine in the secondary treatment used a $C_{avg}t$ product from 200 to 5,000 mg·min/L with applied dose up to 5 mg/L and the contact time up to 1,000 min. To study the effect of water temperature, experiments were conducted at 1, 10 and 22°C. The primary treatment was designed to obtain the same levels of inactivation at each temperature by adjusting the primary chemical dose and contact time. Because hypochlorous acid is the active component in the free chlorine treatment, pH 6 was selected to obtain high concentration of this component when free chlorine was used for the secondary chemical. Monochloramine was relatively stable in a wide pH range, and pH 8 was selected when monochloramine was used in the secondary treatment.

The effect of ozone primary inactivation and the gross kill by the sequential inactivation were evaluated by serial sampling from a single reactor. In the secondary inactivation, samples were taken at different contact times to measure the effect under different Ct products.

3.8 STATISTICS

3.8.1 Maximum-Likelihood Model Parameters Estimation

3.8.1.1 Censored Data Set

The kinetic model parameters were estimated using the maximum-likelihood method by taking the advantage of including some censored data in the model estimation. A logistic likelihood function was given by Haas and Jacangelo (1993) but it can be used for one side censored data only. For a data set with double side censored (left-censored and right-censored), a universal likelihood function was derived as follow.

Assuming the ith observation has a mean value of μ_i, and its error follows the normal distribution with a common standard deviation σ, the probability of $y = y_i$ can be expressed as

$$p(y = y_i | \mu_i, \sigma) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y_i - \mu_i)^2}{2\sigma^2}} \tag{3-16}$$

For left censored data point, the probability of $y \le y_i^{<}$ is the cumulative probability of the standard normal distribution N($\frac{y_i^{<} - \mu_i}{\sigma}$,1) from -∞ to $\frac{y_i^{<} - \mu_i}{\sigma}$

$$p(y \le y_i^{\le} | \mu_i, \sigma) = \int_{-\infty}^{\frac{y_i^{\le}-\mu_i}{\sigma}} \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}} dx = \Phi(\frac{y_i^{\le}-\mu_i}{\sigma}) \qquad (3\text{-}17)$$

$$\Phi(z) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{z} e^{-\frac{x^2}{2}} dx$$

where $y_i^{\le}$ = the detection limit for the left-censored trial ith; and $\Phi(z)$ = the cumulative standard normal distribution, For right censored data point, the probability of $y \ge y_i^{\ge}$ is the cumulative probability of the standard normal distribution $N(\frac{y_i^{\ge}-\mu_i}{\sigma}, 1)$ from $\frac{y_i^{\ge}-\mu_i}{\sigma}$ to $+\infty$

$$p(y \ge y_i^{\ge} | \mu_i, \sigma) = \int_{\frac{y_i^{\ge}-\mu_i}{\sigma}}^{+\infty} \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}} = 1 - \int_{-\infty}^{\frac{y_i^{\ge}-\mu_i}{\sigma}} \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}} dx = 1 - \Phi(\frac{y_i^{\ge}-\mu_i}{\sigma}) \qquad (3\text{-}18)$$

where $y_i^{\ge}$ = the detection limit for the right-censored trial ith. The overall probability of all the data set gives the likelihood function

$$L_o = \prod_{i=1}^{v_o} \frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{(y_i-\mu_i)^2}{2\sigma^2}} \prod_{i=1}^{v_1} \Phi(\frac{y_i^{\le}-\mu_i}{\sigma}) \prod_{i=1}^{v_2} (1-\Phi(\frac{y_i^{\ge}-\mu_i}{\sigma})) \qquad (3\text{-}19)$$

Ignoring the constants, the natural logarithm of the likelihood function can be given by

$$\ln(L_o) = -v_o \ln\sigma - \frac{1}{2}\sum_{i=1}^{v_o} (\frac{y_i-\mu_i}{\sigma})^2 + \sum_{i=1}^{v_1} \ln\Phi(\frac{y_i^{\le}-\mu_i}{\sigma}) + \sum_{i=1}^{v_2} \ln(1-\Phi(\frac{y_i^{\ge}-\mu_i}{\sigma})) \qquad (3\text{-}20)$$

where v_o = the number of non-censored data points; v_1, v_2 = the left-censored and right-censored data points respectively; μ_i = the model prediction value for trial ith; and σ = the standard deviation of the regression errors which can be estimated along with the model parameters. Microsoft Excel 98 (Microsoft Corp.) solver was used to maximize the likelihood function.

3.8.1.2 Non-Censored Data Set

For the non-censored data set, the likelihood function can be simplified as

$$\ln(L_o) = -v_o \ln\sigma - \frac{1}{2}\sum_{i=1}^{v_o}\left(\frac{y_i - \mu_i}{\sigma}\right)^2 \qquad (3\text{-}21)$$

3.8.2 Dose-Response Model Parameter Estimation

The logistic dose-response models of CD-1 mice were estimated by the pooled dose-response data set from that batch of oocysts. Model parameters β_o and β_1 can be estimated using the Maximum-Likelihood method by maximizing the likelihood function (Brand et al. 1973)

$$\mathrm{Ln}(L_o) = \sum_{i=1}^{n_d} Y_i(\beta_o + \beta_1 \log(X_i)) - \sum_{i=1}^{n_d} \mathrm{Ln}(1 + \exp(\beta_o + \beta_1 \log(X_i))) \qquad (3\text{-}22)$$

where Y_i = the positive (Y_i =1) or nagtive (Y_i =0) of the mice; i = 1, 2, ... , to n_d , the number of mice used for the dose-response.

3.8.3 Marginal Confidence and Joined Confidence Limits

Marginal confidence intervals and joined confidence interval were calculated for model estimation. The joined confidence interval for 90% confidence level were computed for each of the parameters using the likelihood ratio test (Seber and Wild 1989)

$$\ln L_o(\hat{\beta}) - \ln L_o(\beta_{1-\alpha}) \le \frac{\chi^2_{p,\alpha}}{2} \tag{3-23}$$

where p = the number of parameter; vector $\hat{\beta}$ = the optimal value of the model parameter and vector $\beta_{1-\alpha}$ = the 100(1-α)% marginal limits for the model parameters; and $\chi^2_{p,\alpha}$ = the Chi-square distribution. The marginal confidence limits for a single parameter are also calculated by Equation 3-23 while other parameters are in the optimal prediction value.

3.8.4 Confidence Band for Linear Model

The confidence band of the linear model, y = a + bx, is defined as the area in which lines fitted to repeated experiments of the same kind are expected to lie. Upper and lower (1-α)% confidence level of the model prediction are defined as (Taylor 1990)

$$y_{\text{limit}} = \hat{a} + \hat{b}x \pm (2F^{1-\alpha}_{(2,j-2)})^{1/2} s_y \left[\frac{1}{j} + \frac{(x-\bar{x})^2}{S_{xx}}\right]^{1/2} \tag{3-24}$$

where y_{limit}= upper or lower (1-α)% confidence limit of the model prediction; $\hat{a}$ and $\hat{b}$= optimal estmate of the linear model parameters, a and b, respectively; j = the number of data points used in model parameter estimation; F = Fisher's distribution with (1-α)% confidence level and (2, j-2) degree of freedom; $\bar{x} = \frac{\sum_{i=1}^{j} x_i}{j}$; $S_{xx} = \sum_{i=1}^{j} (x_i - \bar{x})^2$; and

$$s_y = \sqrt{\frac{S_{yy} - \frac{(S_{xy})^2}{S_{xx}}}{j-2}} \tag{3-25}$$

where $S_{yy}=\sum_{i=1}^{j}(y_i-\overline{y})^2$; $\overline{y}=\frac{\sum_{i=1}^{j}y_i}{j}$; and $S_{xy}=\sum_{i=1}^{j}(y_i-\overline{y})(x_i-\overline{x})$. Equation (3-24) can be used to estimate the confidence bands of the CD-1 mice dose-response model (3-1).

3.8.5 Confidence Band for Nonlinear Model

The confidence interval of the kinetic model prediction of log kill was estimated by a linear approximation prediction intervals using Scheffe type (1-α)% confidence bounds and the Hacobian linearization given by Seber (1977)

$$\hat{y}_{x,\hat{\beta}} \pm s\left\{\hat{y}_{x,\hat{\beta}}\right\}\cdot z_{\alpha/2} \tag{3-26}$$

where $\hat{y}_{x,\hat{\beta}}$= predicted survival ratio in the log-kill model; **x** = the condition of the disinfection (concentration and contact time); $\hat{\beta}$ = the optimal model parameter estimate; $Z_{\alpha/2}$ = standard normal distribution (note that for a large data set (n>30) the normal distribution can be used in approximation of the t-distribution); and $s\left\{\hat{y}_{x,\hat{\beta}}\right\}$ = square root of the variance of the model predicted survival ratio, which was given by Seber and Wild (1989) as

$$s^2\left\{\hat{y}_{x,\hat{\beta}}\right\}=s^2\left[1+\hat{\mathbf{f}}_x'(\hat{\mathbf{F}}'.\hat{\mathbf{F}})^{-1}\hat{\mathbf{f}}_x\right] \tag{3-27}$$

For the two parameters (θ and k_{22}) in the temperature corrected Chick-Watson model (Equation 3-11, with n=1)

$$\hat{f}'_x = \left[-\frac{(T-22)\hat{k}_{22}\hat{\theta}^{T-23}}{k'_x}(C_{o_x} - C_{f_x}), -\frac{\hat{\theta}^{T-22}}{k'_x}(C_{o_x} - C_{f_x}) \right] \tag{3-28}$$

For the four parameters (θ, k_{22}, m and n) in the temperature corrected I.g.H. model (Equation 3-13),

$$\hat{f}'_x = \left[\frac{\partial y_{(x;\hat{\theta})}}{\partial \theta}, \frac{\partial y_{(x;\hat{k}_{22})}}{\partial k_{22}}, \frac{\partial y_{(x;\hat{m})}}{\partial m}, \frac{\partial y_{(x;\hat{n})}}{\partial n} \right] \tag{3-29}$$

where $y_{(x;\hat{\theta})}$ = the temperature corrected function at variable **x** with optimal predicted parameters.

For the temperature corrected Chick-Watson model (Equation 3.11), $\hat{\mathbf{F}}$ is two columns × j rows matrix

$$\hat{\mathbf{F}} = \begin{bmatrix} -\frac{(T-22)\hat{k}_{22}\hat{\theta}^{T-23}}{k'_i}(C_{o_i} - C_{f_i}) & -\frac{\hat{\theta}^{T-22}}{k'_i}(C_{o_i} - C_{f_i}) \\ \vdots & \vdots \\ -\frac{(T-22)\hat{k}_{22}\hat{\theta}^{T-23}}{k'_j}(C_{o_j} - C_{f_j}) & -\frac{\hat{\theta}^{T-22}}{k'_j}(C_{o_j} - C_{f_j}) \end{bmatrix} \tag{3-30}$$

where i =1, 2, … j, the number of data points used in the model estimation.

For the temperature corrected I.g.H. model (Equation 3-13), $\hat{\mathbf{F}}$ is four columns × j rows matrix.

$$\hat{\mathbf{F}} = \begin{bmatrix} \frac{\partial \hat{y}_{(\mathbf{x}_1;\hat{\theta},\hat{k}_{22},\hat{m},\hat{n})}}{\partial \theta} & \frac{\partial \hat{y}_{(\mathbf{x}_1;\hat{\theta},\hat{k}_{22},\hat{m},\hat{n})}}{\partial k_{22}} & \frac{\partial \hat{y}_{(\mathbf{x}_1;\hat{\theta},\hat{k}_{22},\hat{m},\hat{n})}}{\partial m} & \frac{\partial \hat{y}_{(\mathbf{x}_1;\hat{\theta},\hat{k}_{22},\hat{m},\hat{n})}}{\partial n} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial \hat{y}_{(\mathbf{x}_j;\hat{\theta},\hat{k}_{22},\hat{m},\hat{n})}}{\partial \theta} & \frac{\partial \hat{y}_{(\mathbf{x}_j;\hat{\theta},\hat{k}_{22},\hat{m},\hat{n})}}{\partial k_{22}} & \frac{\partial \hat{y}_{(\mathbf{x}_j;\hat{\theta},\hat{k}_{22},\hat{m},\hat{n})}}{\partial m} & \frac{\partial \hat{y}_{(\mathbf{x}_j;\hat{\theta},\hat{k}_{22},\hat{m},\hat{n})}}{\partial n} \end{bmatrix} \qquad (3\text{-}31)$$

The model variance-covariance matrix, which indicates the relation of the model parameters, can be calculated as

$$\hat{\Sigma} = s^2[\hat{\mathbf{F}}^T\hat{\mathbf{F}}]^{-1} \qquad (3\text{-}32)$$

Elements of the Jacobian matrix (**F**) can be estimated using the central finite difference approximation (Seber and Wild 1989)

$$\frac{\partial \hat{y}(\mathbf{x};\hat{\theta})}{\partial \theta} \approx \frac{\hat{y}_{(\mathbf{x};\hat{\theta}+\Delta\theta,\hat{k}_{22},\hat{m},\hat{n})} - \hat{y}_{(\mathbf{x};\hat{\theta}-\Delta\theta,\hat{k}_{22},\hat{m},\hat{n})}}{2\Delta\theta} \qquad (3\text{-}33)$$

with $\Delta\theta$ set to a value of 10^{-7}, the square root of the relative machine precision as recommended by Bates and Watts (1988).

3.8.6 Inverse Prediction for Design Criteria Development

The inverse prediction interval was given by the inequality (Seber and Wild 1989).

$$(y_o - \hat{y}_{\mathbf{x}:\hat{\beta}})^2 \le F^{\alpha}_{(1,n-p)} \cdot s^2\left\{\hat{y}_{\mathbf{x}:\hat{\beta}}\right\} \qquad (3\text{-}34)$$

where y_o = target kill; $F^{\alpha}_{(1,n-p)}$ = upper α quantile of Fisher's distribution; $s^2\left\{\hat{y}_{\mathbf{x}:\hat{\beta}}\right\}$ = the least-squares mean square error (MSE), with the expectation (E) given by Box et al. (1978)

$$E(MSE) = s^2 = \frac{\sum_{i=1}^{j}(y_i - \hat{y}_i)^2}{j-p} \tag{3-35}$$

and MSE is related to the maximum likelihood error variance σ^2 given by Neter et al. (1989)

$$s^2 = \frac{j}{j-p}\sigma^2 \tag{3-36}$$

For the estimated variance-covariance matrix (3-32) to be adequate, the likelihood function must be locally linear in the parameters. Firstly, this requires the planar assumption: the expectation surface of the likelihood function is sufficiently flat at the local solution to be replaced by a tangent plane. And secondly, the uniform coordinate assumption: that straight, parallel equispaced lines in the parameter space map into nearly straight, parallel equispaced lines on the expectation surface. Intrinsic and parameter-effects curvature is negligible for model-data sets that satisfy the planar and uniform coordinate assumption, respectively (Donaldson and Schnabel 1987). An analysis of 67 data set-model combinations by Bates and Watts (1988) showed the planar assumption is generally valid and therefore intrinsic curvature is typically very small. This is not true however for parameter-effects curvature, hence the uniform coordinate assumption should be validated. Parameter-effects curvature does tend to decrease as the fit of the model to the data improves (Bard 1974).

The validity of the uniform coordinate assumption, and hence the degree of parameter-effects curvature, and adequacy of the Jacobian-based variance-covariance matrix, can be assessed by constructing profile likelihoods. A nominal 1-α profile likelihood can be constructed for maximum likelihood estimators using the likelihood ratio (3-23). For least-squares estimators, profile likelihoods can be constructed using the ratio-of-variances (Box et al. 1978)

$$SS(\beta) \leq SS(\hat{\beta})\left[1 + \frac{p}{j-p} F^{\alpha}_{(p,j-p)}\right] \tag{3-37}$$

where SS = sum of square of the residual. Although a Bates-Watts parameter-effects curvature array can be computed to identify deviations from model-data set linearity, curvature arrays can give spuriously high values when a model appears satisfactory in all other respects (Seber and Wild 1989). The graphical approach is considered a more informative method (Cook and Goldberg 1986).

3.9 SOLVING I.G.H. MODELS IN MS EXCEL

Direct solution of the incomplete gamma function is available in some mathematical software packages such as MathcadPlus and Maple V. However, a simple way to handle this function is by Microsoft Excel 98, a common software that is widely available.

3.9.1 Calculation of Log-kill

The gamma function $\gamma(\alpha,x) = \int_0^x e^{-x} z^{\alpha-1} dz$, can be formulated in Excel using the combination of incomplete gamma functions

$$\text{GAMMADIST}(n.k'.t,\ m,\ 1,\ \text{true})\times\text{EXP}(\text{GAMMALN}(m)) \qquad (3\text{-}38)$$

Using equation (3-38), the temperature corrected I.g.H. model equation (3-13) is expressed as

$$\log\frac{N}{N_o} = -\frac{m\cdot k_{22}\cdot\theta^{T-22}\cdot C_o^{n}}{(n\cdot k')^{m}}.\text{GAMMADIST}(n\cdot k'\cdot t, m, 1, \text{true}).\text{EXP}(\text{GAMMALN}(m)) \qquad (3\text{-}39)$$

where GAMMADIST() returns the gamma distribution function and GAMMALN() returns the natural logarithm of the gamma function $\Gamma(x)$. Given knowledge of the ozone decay rate, k', the initial ozone residual at time zero (after demand has been satisfied), C_o, and the desired contact time, t (ideal without allowance for non-ideal reactors) and temperature (T), equation (3-39) can be used to estimate the inactivation under the specified conditions.

3.9.2 Calculation of Required Initial Concentration (C_o)

A common practice in drinking water treatment design is to specify a target inactivation level and then to calculate the ozone dose that was required for the system given the temperature, ozone demand and decay data, and the desired contact time. The required initial ozone residual (after demand has been satisfied) was calculated from:

$$C_o = (y\cdot(n\cdot k')^{m}/(m\cdot k_{22}\cdot\theta^{T-22}\cdot\text{GAMMADIST}(n\cdot k'\cdot t, m, 1, \text{true}).\text{EXP}(\text{GAMMALN}(m))))^{\frac{1}{n}} \qquad (3\text{-}40)$$

where y= -log(N/N_o), the target logistic inactivation of *C. parvum* oocysts (log-units).

The designed ozone applied dose then can be determined by the required initial ozone concentration plus the instant ozone demand.

3.9.3 Calculation of Required Contact Time (t)

Another situation arises when the target inactivation level is specified but the contact time needs to be calculated given the temperature, ozone demand and decay data, and the desired initial ozone residual at time zero. The required contact time (ideal plug flow) was calculated from

$$p = y \cdot (n \cdot k')^{m} /(m \cdot k_{22} \cdot \theta^{T-22} \cdot C_{o}^{n} \cdot \mathrm{EXP}(\mathrm{GAMMALN}(m))) \tag{3-41}$$

and

$$t = \mathrm{GAMMAINV}(p, m, 1)/(n \cdot k') \tag{3-42}$$

where GAMMAINV() = the MS Excel function returns the inverse of the cumulative distribution function; and p = the cumulative distribution ($0 \le p \le 1$). It should be noted that for calculation purposes the initial ozone concentration should be high enough to ensure that some ozone residual remains at the end of contact time otherwise an error will occur.

CHAPTER 4

DOSE-RESPONSE AND CONTROLS

4.1 DOSE-RESPONSE

The *C. parvum* oocysts used in this study came from 19 batches. ID_{50} (the infectious dose that caused the infection of 50% of the CD-1 mice cohort) and the logistic dose-response models parameters are summarized in (Table 4-1). The dose-response model parameters, β_o and β_1, were estimated using the maximum likelihood method (3-22) and their 90% confidence limits were estimated by the likelihood ratio test (3-23). The ID_{50} for neonatal CD-1 mice ranged from 41 to 347 oocysts per animal, and for most of batches of oocysts, ID_{50} were less than 100 oocysts per animal. The difference of ID_{50} among different batches of oocysts confirmed that the dose-response was batch specific and individual dose-response model was necessary for each batch of oocysts.

The dose-response models of CD-1 mice gave a very good fit to the experimental data. Figure 4-1 shows the observed data against the model prediction with 90% confidence interval. In the range of interest, the 90% confidence prediction is in a narrow range. Further analysis of the dose-response model showed that the two parameters of the dose-response model, β_o and β_1, were highly confounded, which made the 90% confidence range of the model relatively big (Figure 4-2). From the figure, we can conclude that the 90% joint confidence range of β_o and β_1 for No. 15 batch of oocysts was (-7.8, 4.0) to (-2.3, 1.2), corresponding to the ID_{50} values of 80 to 84 oocysts per animal. Due to the high correlation of β_o and β_1, two batches of oocysts that have the same ID_{50} value may have totally different model parameters. For example, batch No. 13 and 14, which had the ID_{50} values of 63 and 65 oocysts per animal, respectively, had significantly different model parameters (Table 4-1).

Table 4-1. Logistic dose-response models for neonatal CD-1 mice exposed to different batches of *C. parvum* oocysts

Batch No	Number of mice used for the model	β_o^*	90% β_o limits	β_1	90% β_1 limits	ID_{50} (oocysts per mouse)
1	224	-7.96	-8.3, -7.6	4.06	3.9, 4.2	91
2	177	-3.52	-3.9, -3.2	1.88	1.7, 2.1	74
3	133	-5.55	-6.0, -5.1	2.59	2.4, 2.8	139
4	226	-6.02	-6.4, -5.7	2.37	2.2, 2.5	347
5	56	-5.63	-6.2, -5.1	3.17	2.9, 3.5	60
6	139	-7.66	-8.0, -7.3	4.25	4.0, 4.5	64
7	110	-6.34	-6.7, -6.0	2.98	2.8, 3.1	134
8	190	-9.42	-9.7, -9.1	3.97	3.9, 4.1	234
9	189	-7.28	-7.6, -7.0	4.51	4.3, 4.7	41
10	237	-7.15	-7.4, -6.9	4.09	3.9, 4.2	56
11	243	-6.93	-7.2, -6.7	3.67	3.5, 3.8	77
12	40	-4.37	-4.9, -3.8	2.30	2.0, 2.6	79
13	80	-3.12	--3.5, -2.7	1.73	1.6, 1.9	63
14	80	-7.28	-7.7, -6.8	4.01	3.8, 4.3	65
15	110	-4.93	-5.3, -4.6	2.58	2.4, 2.7	82
16	80	-8.65	-9.2, -8.1	4.40	4.1, 4.7	93
17	110	-5.97	-6.3, -5.6	2.91	2.7, 3.1	113
18	175	-5.43	-5.7, -5.1	3.08	2.9, 3.2	58
19	205	-9.89	-10.2, -9.6	5.17	5.0, 5.3	82

* Dose-response model is expressed as Equation 3-1

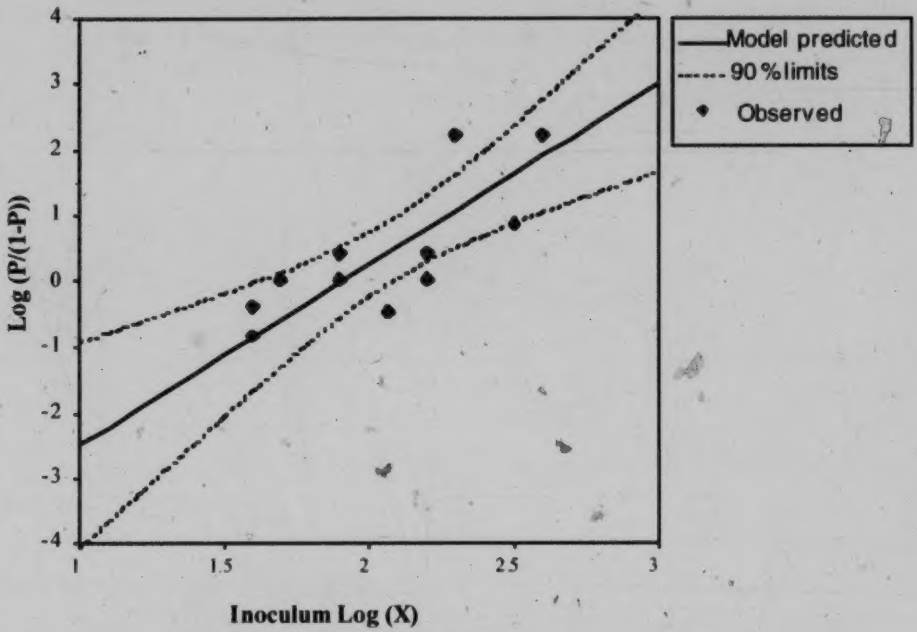


Figure 4-1. Model prediction with 90% confidence interval for CD-1 mice dose-response to *C. parvum* oocysts (batch No. 15)

In a previous study (Finch et al. 1993b) of *C. parvum* infection in neonatal CD-1 mice, an ID $_{50}$ of 79 oocysts per animal was reported. Korich et al. (2000) compared the CD-1 neonatal mouse logistic dose-response model for *C. parvum* oocysts, and showed that the dose-response models obtained from two different laboratories for the same Iowa strain of *C. parvum* oocysts were statistically the same. ID$_{50}$ values were from 60 to 87 oocysts per animal. Their models were also statistically the same as that reported by Finch et al. (1993b).

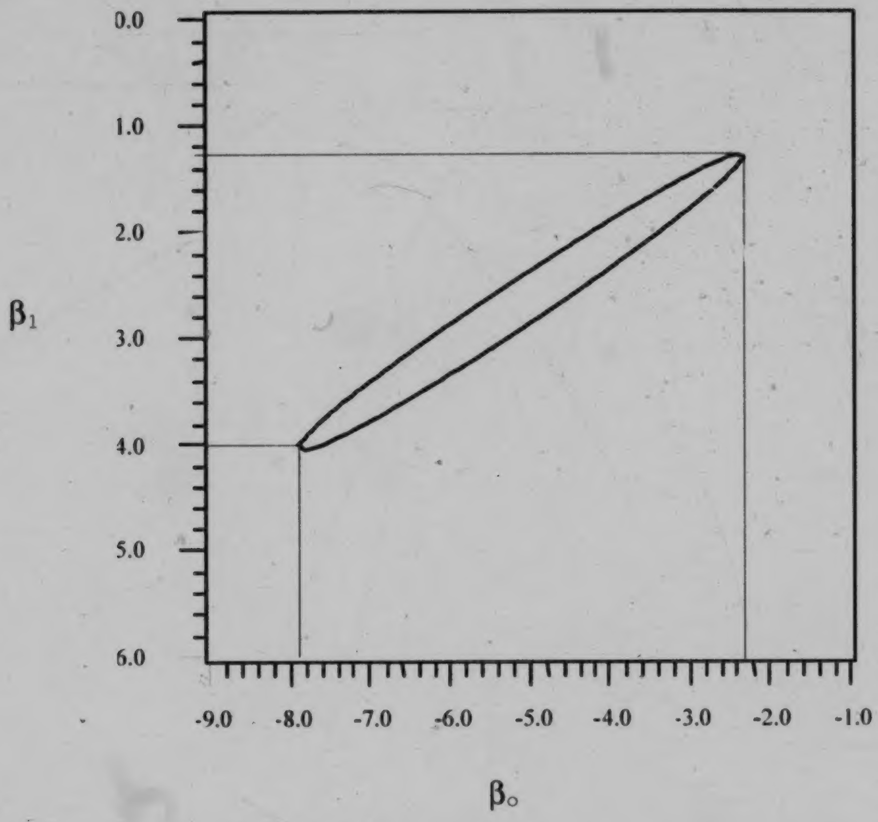


Figure 4-2. 90% Joint confidence interval (JCR) of CD-1 mice dose-response model parameters, β_o and β_1, for batch No. 15 *C. parvum* oocysts

4.2 CONTROL

The control trials in this study were conducted in a period of three years and the results are analyzed separately. Details of the control trials are summarized in Appendix C. The reduced infectivity in the control trials varied from -0.8 to 0.9 log-units, mostly within ±0.5 log-units. The reduction in infectivity of the oocysts in the controls for were 0.4±0.03 log-units (n=58) in average. A histogram showing the reduction in infectivity of the oocysts in the controls is presented in Figure 4-3.

Under the experimental conditions, ambient temperature was found not to have significant effect on the oocyst infectivity (Fayer 1994a; Fayer and Nerad 1996). The quench reagents and the recovery process also did not affect oocyst infectivity (Liyanage et al. 1997a). As a result, the control trials served as a quality assurance for the experiments.

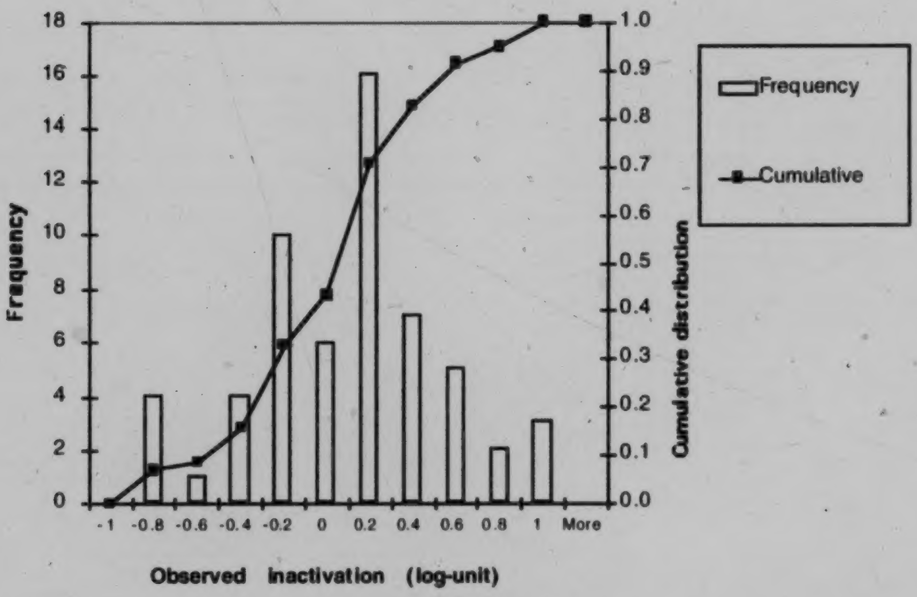


Figure 4-3. Histogram of the reduced infectivity of *C. parvum* oocysts in the control trials in oxidant demand-free 0.05 M phosphate buffer

CHAPTER 5

OZONE

5.1 INTRODUCTION

Ozone was found to be the most effective single chemical for *C. parvum* oocyst inactivation (Peeters et al. 1989; Korich et al. 1990b; Finch et al. 1994b). The kinetics of ozone inactivation of *C. parvum* oocysts at room temperature under laboratory conditions have been well documented (Gyürék et al. 1999). Preliminary results of ozone inactivation of *C. parvum* oocysts at low temperature showed that the efficacy of ozone was reduced by 50% for every 10°C decrease in temperature (Finch and Li 1999).

In this chapter, ozone inactivation of *C. parvum* oocysts at different temperatures and pH values is reported using animal infectivity as the indicator of microbial reduction. Ozone concentration and contact time requirement at different temperatures are calculated based on a fitted rate law describing the inactivation kinetics.

5.2 EXPERIMENTAL SETTINGS

To obtain optimal model parameter estimates, experimental settings for ozone dose and contact time were specified using experimental design criteria described by Box and Lucas (1959). The ozone dose ranged from 0.3 to 2.8 mg/L with contact time from 4 to 30 min. Some pseudo-replicates were collected, and experiments at different temperature and pH settings were run in random order.

To examine the effect of pH on ozone inactivation kinetics, experiments at 1°C and pH 6, 7 and 8 were collected as seven paired tests. To estimate the temperature coefficient, θ, data were collected at two different temperatures (1 and 22°C). Data were also collected at 5 and 13°C to validate the quality of fit of the van't Hoff-Arrhenius model for temperature correction. As well, two data points at 37°C (different ozone doses and contact times) were collected to verify model fit at higher temperatures.

5.3 RESULTS AND DISCUSSION

5.3.1 Microbial Reduction Following Ozone Treatment

Experimental data for ozone inactivation at 1°C and 22°C and pH 6 to 8 are summarized in Table 5-1 and Table 5-2. The reactor volume used in this study was 250 or 500 mL with the parasite number ranging from 2.5×10^7 to 1.0×10^8 oocysts, depending on the target inactivation levels. At 1°C, the doses and contact times required for the same levels of inactivation were much higher than those at 22°C. At 1°C, the ozone doses ranged from 0.9 to 2.8 mg/L and the contact times from 4 to 30 min, with the observed kill from -0.2 to 3.0 log-units. In the paired tests between pH 6 and 8 using the same dose and contact time, comparable kills were observed at different pH using the same ozone doses and contact times (*e.g.* Trial No. 601 and 602, 647 and 648). At 1°C, ozone decay at pH 8 was slightly higher than at pH 6 and 7.

Table 5-1. Summary of ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 at 1±0.5°C

Trial No.	pH	Initial ozone residual C_o (mg/L)	Final ozone residual C_f (mg/L)	Contact time t (min)	First order ozone decay rate constant (1/min)	Reactor volume (mL)	Number of oocysts in reactor	Reduction in infectivity (log-units)	I.g.H. model predicted reduction in infectivity* (log-units)
601	6	1.0	0.9	4	0.03	200	5.0×10^7	-0.2	0.4
652	6	1.0	0.9	5	0.02	200	2.5×10^7	0.4	0.4
672.1	6	2.3	1.8	10	0.02	500	6.0×10^7	1.3	1.2
671.1	6	1.0	0.9	15	0.01	500	6.0×10^7	0.6	0.9
670.1	6	1.8	1.5	15	0.01	500	6.0×10^7	1.6	1.4
603	6	1.0	0.2	17	0.09	200	2.5×10^7	0.8	0.7
631.1	6	0.8	0.4	30	0.02	200	1.0×10^8	0.8	1.1
647	6	1.0	0.5	30	0.02	200	2.5×10^7	1.2	1.3
665.1	6	1.9	1.5	30	0.01	500	6.0×10^7	2.4	2.3
666.1	6	1.9	1.5	30	0.01	500	6.0×10^7	2.5	2.3
579	6	2.7	1.7	30	0.01	200	5.0×10^7	3.0	2.8
557	7	0.9	0.8	4	0.03	200	5.0×10^7	0.2	0.3
558	7	2.0	1.8	4	0.03	200	5.0×10^7	0.7	0.6
544	7	2.5	2.2	10	0.01	200	5.0×10^7	1.6	1.3
543	7	1.2	0.9	20	0.01	200	5.0×10^7	1.2	1.2
548	7	0.8	0.5	30	0.02	200	5.0×10^7	1.0	1.1
545	7	2.8	1.7	30	0.02	200	5.0×10^7	2.7	2.8
602	8	1.0	0.9	4	0.03	200	5.0×10^7	-0.2	0.4
653	8	1.1	0.8	5	0.06	200	2.5×10^7	0.4	0.4
707	8	0.9	0.5	15	0.03	200	1.0×10^8	0.2	0.7
677.1	8	1.0	0.7	15	0.02	500	5.0×10^7	0.4	0.8
604	8	1.0	0.3	20	0.06	200	2.5×10^7	0.2	0.8
648	8	0.9	0.3	30	0.04	200	2.5×10^7	0.7	1.0
632.1	8	0.9	0.3	30	0.04	200	1.0×10^8	1.5	1.0
706	8	1.4	0.5	30	0.03	200	1.0×10^8	1.4	1.5
580	8	2.7	1.3	30	0.02	200	5.0×10^7	2.7	2.6

* Model is expressed as Equation 3.13 and parameters are listed in Table 5-4

Table 5-2. Summary of ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 at 22±1°C

Trial No.	pH	Initial ozone residual C_o (mg/L)	Final ozone residual C_f (mg/L)	Contact time t (min)	First order ozone decay rate constant (1/min)	Reactor volume (mL)	Number of oocysts in reactor	Reduction in infectivity (log-units)	I.g.H. model predicted reduction in infectivity* (log-units)
319	6	0.7	0.7	4	0.01	1000	3.0×10^{7}	2.1	1.4
502	6	0.5	0.4	5	0.04	250	5.0×10^{7}	1.6	1.2
644	6	0.9	0.7	5	0.05	250	5.0×10^{7}	2.2	1.8
503	6	1.9	1.7	5	0.02	250	5.0×10^{7}	3.2	3.3
610	6	0.9	0.7	10	0.03	250	5.0×10^{7}	2.6	3.0
504	6	0.5	0.3	20	0.03	250	5.0×10^{7}	3.3	3.0
225	7	0.6	0.2	3	0.44	50	3.5×10^{5}	0.5	0.8
117	7	1.2	0.4	4	0.27	50	5.0×10^{6}	1.3	1.5
25	7	0.7	0.3	4.5	0.19	50	3.6×10^{7}	1.9	1.2
11	7	2.4	1.2	5	0.14	50	1.0×10^{6}	3.6	3.3
26	7	2.4	0.5	9.5	0.17	50	3.6×10^{7}	4.1	4.2
27	7	1.5	0.2	10	0.18	50	1.1×10^{7}	3.4	2.8
24	7	1.7	0.3	10	0.17	50	1.0×10^{6}	2.4	3.3
10	7	1.7	0.3	10	0.17	50	1.0×10^{6}	3.5	3.3
509	7	1.1	0.7	15	0.03	250	5.0×10^{7}	4.0	4.4
28	7	2.2	0.2	15	0.16	50	1.1×10^{7}	4.6	4.6
508	7	0.7	0.2	30	0.04	250	5.0×10^{7}	3.9	4.2
118	8	1.4	0.2	4	0.45	50	5.0×10^{6}	1.1	1.5
123	8	1.5	0.3	4	0.40	50	5.0×10^{6}	1.1	1.6
332	8	1.7	0.3	4.5	0.36	250	7.5×10^{6}	1.1	1.8
645	8	0.9	0.4	5	0.16	250	5.0×10^{7}	1.4	1.6
642	8	0.8	0.2	10	0.14	250	5.0×10^{7}	1.4	2.0
611	8	0.9	0.6	10	0.04	250	5.0×10^{7}	2.8	2.9
553	8	1.1	0.1	14	0.17	250	5.0×10^{7}	2.6	2.6

* Model is expressed as Equation 3.13 and parameters are listed in Table 5-4

At 22°C, data were collected using initial ozone doses from 0.5 to 2.4 mg/L and contact times from 3 to 30 min. The observed kills were 0.25 to 4.6 log-units. The ozone decays at pH 7 and pH 8 were much higher than those at pH 6 (Table 5-2) partly due to the higher ozone auto-decomposition at higher pH. However, one important factor that contributed to the high ozone decay was the small reactor volume. Experiments showed that the ozone decay in a 50 mL reactor was several times in a reactor of volume greater than 200 mL.

Data collected at pH 7 at 5, 13 and 37°C are summarized in Table 5-3. The data collected at different temperatures clearly showed the dramatic temperature impact on

Table 5-3. Summary of ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 7 at 5, 13 and 37°C

Trial No.	Temp (°C)	Initial ozone residual C_o (mg/L)	Final ozone residual C_f (mg/L)	Contac t time t (min)	First order ozone decay rate constant (1/min)	Reactor volume (mL)	Number of oocysts in reactor	Reduction in infectivity (log-units)	I.g.H. model predicted reduction in infectivity * (log-units)
568.3	5	1.9	1.8	2	0.03	500	1.0×10^8	0.4	0.5
568.4	5	1.9	1.6	4.3	0.04	500	1.0×10^8	1.1	0.8
534	5	1.2	0.8	20	0.02	200	5.0×10^7	1.8	1.6
568.6	5	1.9	0.7	20	0.05	500	1.0×10^8	2.1	1.9
571.2	13	1.0	0.9	2	0.05	500	1.0×10^8	-0.2	0.5
571.3	13	1.0	0.7	5	0.07	500	1.0×10^8	1.0	1.0
570	13	1.8	1.7	4	0.01	200	5.0×10^7	2.0	1.4
571.4	13	1.0	0.4	15	0.06	500	1.0×10^8	2.0	1.8
569	13	1.1	0.6	20	0.03	200	5.0×10^7	3.0	2.6
571.5	13	1.0	0.2	23	0.07	500	1.0×10^8	2.2	2.1
582	37	0.3	0.1	4.0	0.27	200	5.0×10^7	2.7	1.8
581	37	0.7	0.4	4.0	0.14	200	5.0×10^7	4.0	3.8

* Model is expressed as Equation 3.13 and parameters are listed in Table 5-4

ozone inactivation of *C. parvum* oocysts. At 5°C, an ozone dose of 1.9 mg/L for 20 min ($C_{avg}t$ =27 mg·min/L) resulted in an inactivation of 2.1 log-units, while at 13°C, an ozone dose of 1 mg/L for 15 min ($C_{avg}t$ =10.5 mg·min/L) resulted in an inactivation of 2.0 log-units. As the water temperature increased, the oocysts were more readily inactivated. At 37°C, an ozone dose of 0.8 mg/L for 4 min ($C_{avg}t$ =2.3 mg·min/L) resulted in an inactivation of 4.0 log-units. Thus, Ct requirement for a given level of kill decreased dramatically with an increase in water temperature.

In trials where very low ozone dose was used, such as 1 mg/L for 4 min at 1°C (*i.e.* Trial No. 602) and 1 mg/L for 2 min at 13°C (*i.e.* Trial No. 571.2), the observed kill using animal infectivity has a negative value. This was not indicative of enhanced infectivity but rather of the inherent variability of the animal infectivity assay.

5.3.2 Microorganism Reduction Kinetics

Oocysts survival curve after ozonation at 1 to 37°C is illustrated at Figure 5-1. $C_{avg}t$ instead of the conventional Ct product (C = the final concentration) was used to account for the ozone decay. The oocyst survival curves were characterized by an apparent lag followed by a fast decline, then an evident tail-off. The shoulder and tail-off effect were more evident at low temperatures. The non-linear nature of the survival curves suggested that a non-linear model should be used for the microorganism reduction kinetics.

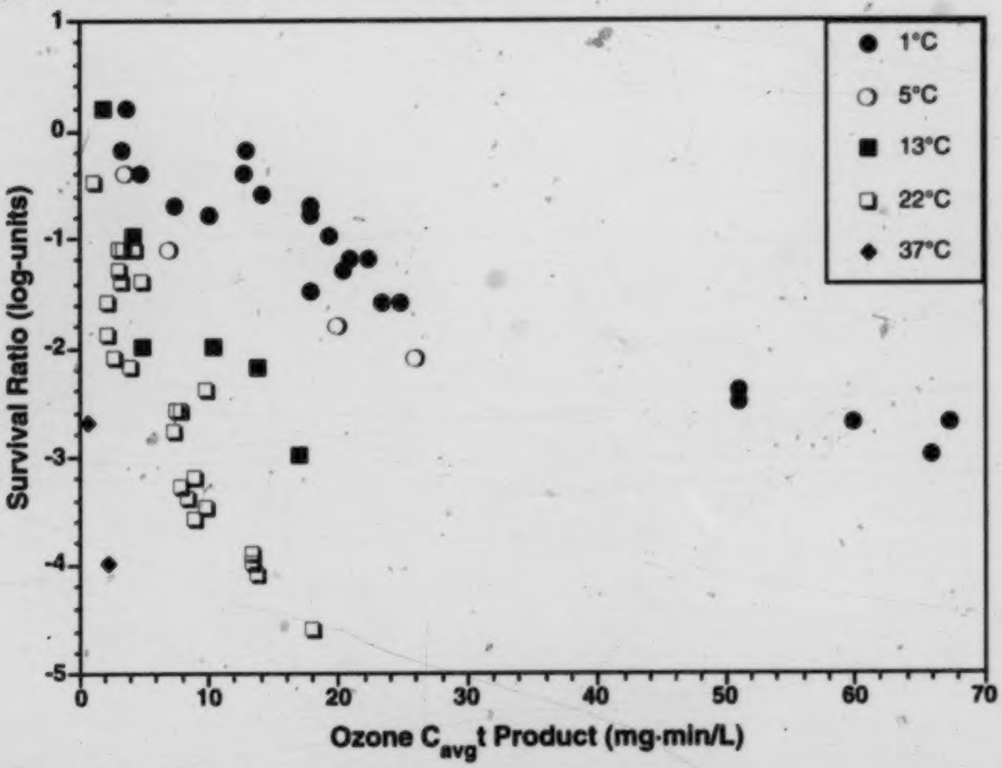


Figure 5-1. Survival ratios of *C. parvum* oocysts as the function of $C_{avg}t$ products after ozone treatment in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 and 1 to 37°C

The I.g.H. model parameters for 1°C and pH 6 to 8 are listed in Table 5-4. When compared to those at 22°C (Gyürék et al. 1999), the n and m values at these two temperatures were very similar (at 1°C, $\hat{n}$=0.93, $\hat{m}$=0.78 and at 22°C $\hat{n}$=0.73, $\hat{m}$=0.70) and the 90% confidence limits of n overlapped. However, the inactivation rate constant, k, at 22°C was 6.8 times that at 1°C ($\hat{k}_1$=0.10 and $\hat{k}_{22}$=0.68). These findings suggested that a single I.g.H. model with the same n and m values may be applied for different temperatures, provided the inactivation rate constant is adjusted appropriately.

An I.g.H. model that accounts for the first order ozone decay was calibrated for 1 to 37°C and pH 6 to 8. Model parameters and their 90% confidence limits are

Table 5-4. Model parameters for ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 and 1 to 37°C.

Kinetic model	I.g.H. *				Chick-Watson †
Temperature (°C)	1	22	1 to 37	1 to 37	1 to 37
pH	6, 7, 8	6, 7, 8	7	6, 7, 8	6, 7, 8
Number of trials	26	24	29	62	62
Temperature correction, $\hat{\theta}$ (±90% limits)	-	-	1.07 (1.06, 1.08)	1.08 (1.07, 1.09)	1.10 (1.10, 1.11)
$\hat{k}$ (±90% limits)	0.10 (1°C) (0.09, 0.11)	0.68 (22°C) (0.62, 0.73)	0.89 (22°C) (0.85, 0.92)	0.68 (22°C) (0.65, 0.70)	0.39 (22°C) (0.36, 0.41)
$\hat{m}$ (±90% limits)	0.78 (0.76, 0.80)	0.70 (0.66, 0.74)	0.59 (0.57, 0.61)	0.71 (0.70, 0.73)	
$\hat{n}$ (±90% limits)	0.93 (0.83, 1.02)	0.73 (0.55, 0.92)	0.61 (0.53, 0.70)	0.73 (0.65, 0.81)	
error, σ	0.26	0.43	0.35	0.38	0.48
Model constraints: Initial residual C_o (mg/L) Contact time t (min)	$0.2 \le C_o \le 2.8$ $5 \le t \le 30$	$0.1 \le C_o \le 2.4$ $3 \le t \le 30$	$0.1 \le C_o \le 2.4$ $3 \le t \le 30$	$0.1 \le C_o \le 2.4$ $2 \le t \le 30$	$0.1 \le C_o \le 2.4$ $2 \le t \le 30$

* Equation 3-7 for single temperature and Equation 3-13 for multiple temperature;
† Equation 3-11.

shown in Table 5-4. The fitness plot showed that the model gave a very good prediction for different temperatures (Figure 5-2a). The model parameters given by the pH 6 to 8 data and the pH 7 data alone were very similar. These results confirmed that, when the difference of ozone decay was accounted for, water pH was not an important factor for the ozone inactivation of *C. parvum* oocysts. The n and m values for the model were very similar ($\hat{n}$=0.71 and $\hat{m}$=0.73), suggesting that the concentration and contact time were equally important for ozone inactivation of the parasites.

A Chick-Watson model accounting for the first order ozone decay was also calibrated using the data set at pH 6 to 8 and 1 to 37°C (Table 5-4). This linear model did not fit the survival curves that have an evident lag and long tail-off. It under-predicted the kill for the range about 1.0 to 2.5 log-units of inactivation and over-predicted the kill at higher than 3.0 log-units of inactivation. Thus, the non-linear I.g.H. model was more appropriate for prediction of ozone inactivation of *C. parvum* oocysts.

5.3.3 pH Effect

The effect of water pH on ozone inactivation was not significant at low temperatures at pH 6 to 8. For example, an ozone dose of 1 mg/L for 30 min at pH 6, 7 and 8 resulted in an observed kill of 1.2, 1.0 and 1.5, respectively (Table 5-1, Trial No. 647, 548 and 632.1). The paired t-test between pH 6 and 8 indicated that the kills caused by the same ozone dose and contact time at pH 6 or 8 were statistically the same.

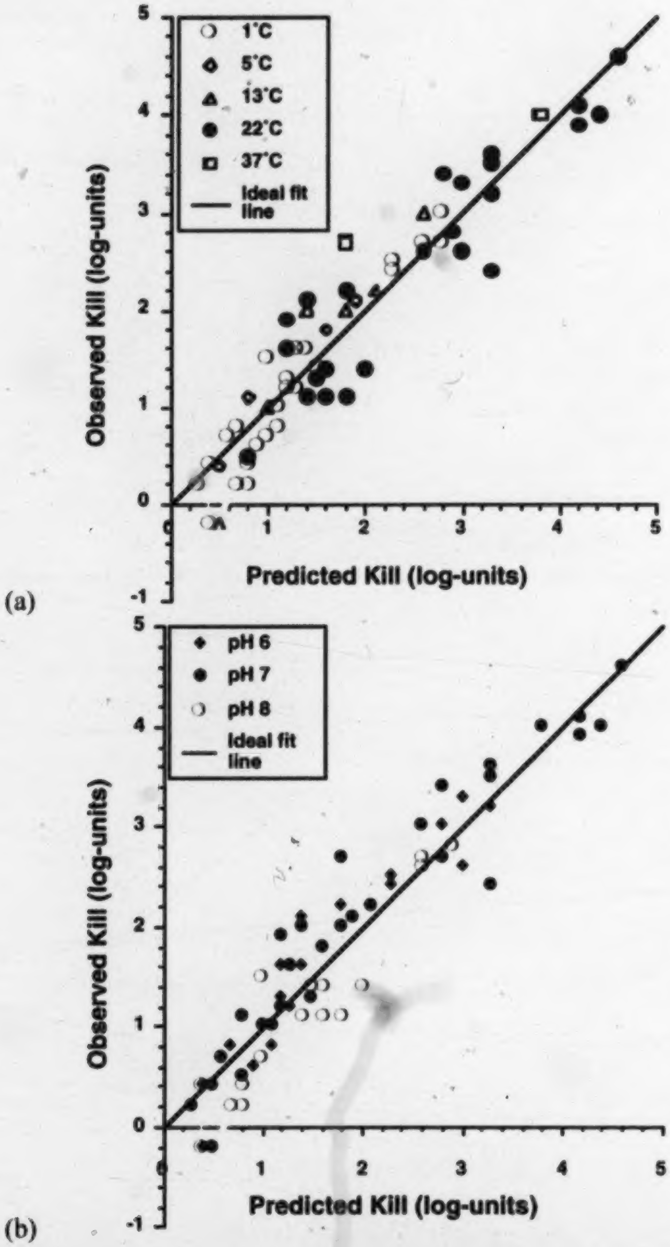


Figure 5-2. Temperature corrected I.g.H. model fit for ozone inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 and 1 to 37°C: a) as a function of temperature, and b) as a function of pH

A study (Gyürék et al. 1999) on ozone inactivation of *C. parvum* oocysts at room temperature showed that, when the difference of ozone decay at different pH was accounted for, pH was not a significant factor for ozone inactivation in the pH range of 6 to 8. These results at low temperatures also suggested that pH was not a significant factor in ozone inactivation of *C. parvum*, indicating that a single model may be used to predict the ozone inactivation at pH range between 6 and 8. A plot of the I.g.H. model fit as a function of pH (Figure 5-2b) showed that the fitted model gave a good prediction for different pH values.

The concentration of hydroxyl radical, an important intermediate product in ozonation that has higher oxidation potential than molecular ozone itself, increases at high pH (Hoigné and Bader 1976). The hydroxyl radical has been shown to be more effective than ozone in the destruction of some recalcitrant taste and odor compounds (Wolfe et al. 1989a). However, since the half-life of this hydroxyl radical is very short and its oxidation reaction is not selective, its ability to inactivate pathogens is limited. Wolfe et al. (1989a; 1989b) reported that, under the same ozone Ct value, the inactivation of *E. coli* and *G. muris* by PEROZONE or ozone alone was comparable. Wickramanayake (1984b) studied ozone inactivation of protozoan cysts at 25°C using semi-batch reactor with continuous ozone supply, and reported theat the pH was not a significant factor in ozone inactivation of *Naegleria gruberi* cysts. Interestingly, Wickramanayake et al. (1984b) reported that ozone was more effective at pH 9 than at lower pH in the inactivation of *G. muris* cysts. Farooq et al. (1977) studied ozone inactivation of *Mycobacterium fortuitum*, and reported that, when the difference in ozone decay was accounted for, the ozone efficacy was relatively stable within pH 5.7 to 10.1.

5.3.4 Temperature Effect

The empirical constant for temperature adjustment, $\hat{\theta}$=1.08, was given by the I.g.H. model at 1 to 37°C. For every 10°C temperature increase the reaction rate constant, k, increased 2.2 times. These results agree with the classic thermodynamics where the reaction rate constant roughly doubles for every 10°C temperature increase. The activation energy, E_a= 52 kJ/mol, and the frequency factor, A= 9.9×10^8 1/min, for ozone inactivation were defined in this study.

The activation energies for ozone inactivation of different organisms are summarized in Table 5-5. Rennecker et al. (1999) reported that for ozone inactivation of *C. parvum* oocysts at pH 7 at 4 to 30°C, the activation energy was 81.2 kJ/mol, which was 58% higher than that from this study. This discrepancy in the findings may due to the different approaches of viability assessment. Rennecker et al. (1999) used *in vitro* excystation, which is a less reliable assay than animal infectivity (Neumann et al. 2000a). The activation energy for ozone inactivation of *N. gruberi* cysts at pH 7 was 31.4 kJ/mol (Wickramanayake and Sproul 1988). Based on the ozone Ct requirement for 99% inactivation at 5°C and 25 °C at pH 7 (Wickramanayake et al. 1985), the activation energies for *G. muris* and *G. lamblia* were estimated as 70.0 and 40.5 kJ/mol,

Table 5-5. Activation energies for ozone inactivation of microorganisms

Microorganisms	E_a (kJ/mol)	pH	Temperature (°C)	Reference
C. parvum oocysts *	52	6 to 8	1 to 37	This study
C. parvum oocysts †	81.2	7	5 to 30	Rennecker et al. (1999)
Escherichia coli	37.1	6	5 to 20	Hunt and Marinas (1997)
Naegleria gruberi cysts	31.4	7	5 to 30	Wickramanayake and Sproul (1988)
Giardia muris ‡	70	7	5 to 25	Wickramanayake et al. (1985)
Giardia lambia ‡	40	7	5 to 25	Wickramanayake et al. (1984a)
Poliovirus I	15.0	7.2	5 to 20	Roy et al. (1981)

* Based on animal infectivity and I.g.H. model;

† Based on *in vitro* excystation and pseudo-first order model;

‡ Estimated from Ct requirement for 99% kill.

respectively. Roy et al. (1981) reported that the activation energy for ozone inactivation of Poliovirus I at pH 7.2 was 15.0 kJ/mol.

5.4 OZONE DISINFECTION DESIGN CRITERIA

Ozone design criteria for different temperatures were developed in this study. For a given level of inactivation, initial ozone concentrations and contact times were calculated using the temperature-corrected I.g.H. model. The required initial ozone concentration and contact time for 1.0 and 2.0 log-units of inactivation was illustrated in

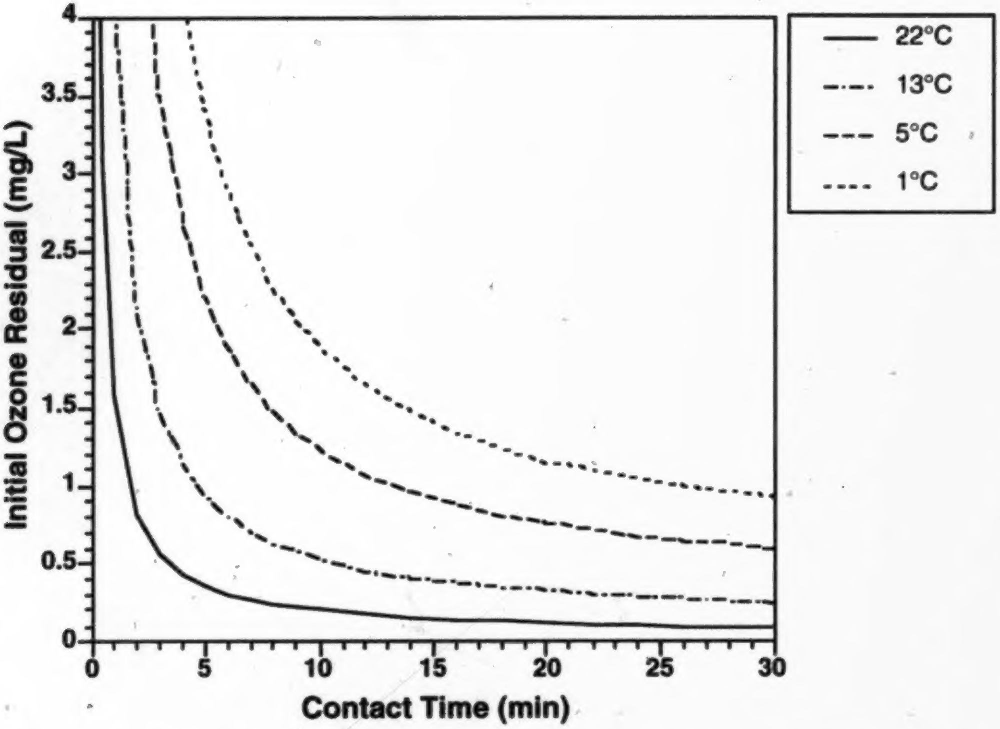


Figure 5-3. Ozone dose and contact time for 1.0 log-unit inactivation of *C. parvum* oocysts predicted by I.g.H. model at pH 6 to 8 and 1 to 22°C, assuming first-order ozone disappearance rate constants of 0.05 1/min and buffered oxidant demand-free water

Figure 5-3 and Figure 5-4. Assuming the ideal plug flow reactor and constant ozone residual concentration, the ozone Ct requirement for 1.0, 2.0, 3.0 log-units of inactivation given by the temperature corrected I.g.H. model at 1, 5, 13 and 22°C are provided at Table 5-6. A minimum ozone concentration of 0.5 mg/L and minimum

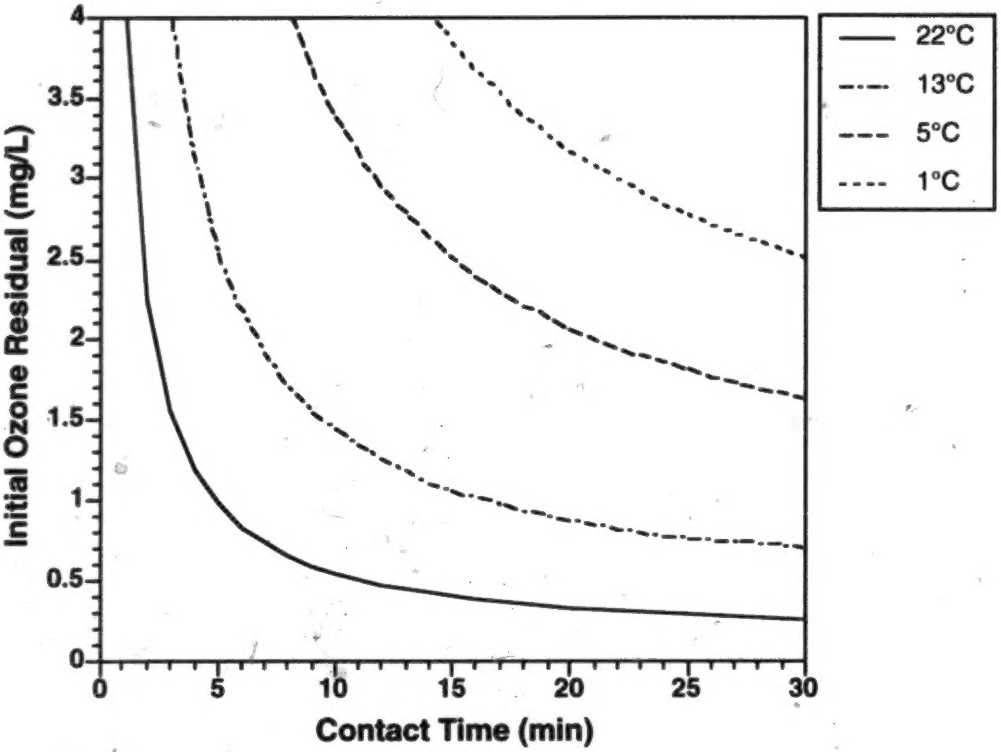


Figure 5-4. Ozone dose the contact time for 2.0 log-units inactivation of *C. parvum* oocysts predicted by I.g.H. model at pH 6 to 8 and 1 to 22°C, assuming first-order ozone disappearance rate constants of 0.05 1/min and buffered oxidant demand-free water

contact time of 5 min were recommended for the design criteria. Because the ozone concentration and contact time are equally important for *C. parvum* oocyst inactivation, the same level of kill could be obtained using a high ozone dose and shorter contact time

Table 5-6. Ct requirement for ozone inactivation of *C. parvum* oocysts at pH 6 to 8, given by I.g.H. model, assuming a constant ozone residual (no safety factor)

Temperature	1±0.5°C	5±0.5°C	13±0.5°C	22±1°C
Target inactivation (log-units)	Ct requirement (mg·min/L)			
1.0	15.3 to 17.0	10.6 to 11.2	4.6 to 4.8	1.8 to 1.9
2.0	41.9 to 43.1	28.0 to 28.9	11.8 to 12.4	4.6 to 4.7
3.0	76.4 to 76.7	49.5 to 50.3	20.8 to 21.7	8.0 to 8.3
Constraint of C (mg/L) and t (min)	$0.5 \leq C \leq 3.0$ $5 \leq t \leq 30$	$0.5 \leq C \leq 3.0$ $5 \leq t \leq 30$	$0.5 \leq C \leq 3.0$ $5 \leq t \leq 30$	$0.5 \leq C \leq 3.0$ $5 \leq t \leq 30$

or low ozone dose and longer contact time. The required Ct for a given inactivation was not a single value since the n and m in the I.g.H. model were not unity. Due to the non-linear nature of the ozone inactivation kinetics, the Ct product for 2.0 log-units of inactivation was more than double the Ct product required for 1.0 log-unit of inactivation.

An ozone Ct product of 18.8 mg·min/L was reported by Ransome et al. (1993) for 98.6% (1.85 log-units) of *C. parvum* inactivation at pH 7 and 10°C. This Ct requirement given by excystation evaluation was slightly higher than that from this study (15 to 16 mg·min/L for the same kill at 10°C). Owen et al. (1995) conducted a pilot scale study of ozone inactivation of *C. parvum* in filtered water at room temperature (22 to 25°C). They reported that a Ct product of 5.5 mg·min/L was required for 99% (2.0 log-units) inactivation of *C. parvum* oocysts, which was similar to the findings of this study. Rennecker et al. (1999) reported that the required Ct for 1.0 log-units inactivation of *C. parvum* oocysts by ozone at 0.5°C was 32.7 mg·min/L, which was twice as high as that predicted by the I.g.H. in this study. Studies by Black et al. (1996) and Owens et al. (1994) using both *in vitro* excystation and animal infectivity assessment showed that the *in vitro* method tended to under estimated the kill of *C. parvum* oocysts. Neumann et al. (2000a) showed that the intact oocysts following an *in vitro* excystation procedure were highly infectious to mice. Thus, their reported finding underscores the potential lack of accuracy associate with excystation-based disinfection studies involving *C. parvum*.

Although the pH did not have a significant impact on the reaction rate constant for ozone inactivation, it may affect the initial ozone demand and ozone decay rate. As a result, a higher ozone dose may be required at higher pH values. Low temperature is a real challenge for ozone inactivation processes. The log-kills corresponding to a typical ozone residual of 1.5 mg/L at different temperatures are illustrated in Figure 5-5, from which we can see that below 5°C, 3.0 log-units of inactivation could not be achieved in 30 min.

A safety factor should be used when the ozone Ct requirements provided in Table 5-6 are applied in the design. An alternative approach incorporating a safety factor in the design was to determine the Ct requirement for the given level of statistical confidence. This can be calculated from data used to estimate the model parameters. Design curves

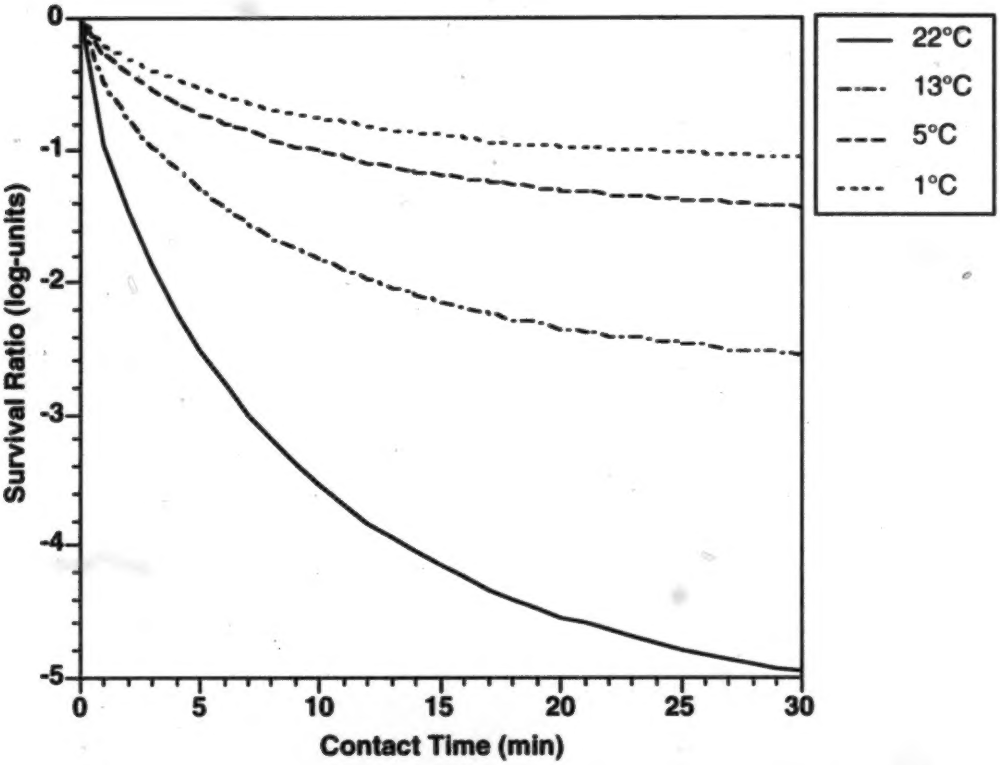


Figure 5-5. I.g.H. model predicted survival curves of *C. parvum* oocysts under 1.5 mg/L initial ozone treatment at different temperatures, assuming first-order ozone disappearance rate constants of 0.05 1/min and buffered oxidant demand-free water

for 1.0 and 2.0 log-units inactivation with 90% confidence level are shown in Figure 5-6 and Figure 5-7. The first-order rate constant of ozone disappearance was assumed as 0.05 1/min in the calculation. The 90% upper confidence interval design dose was about twice the mean expected dose given by I.g.H. model.

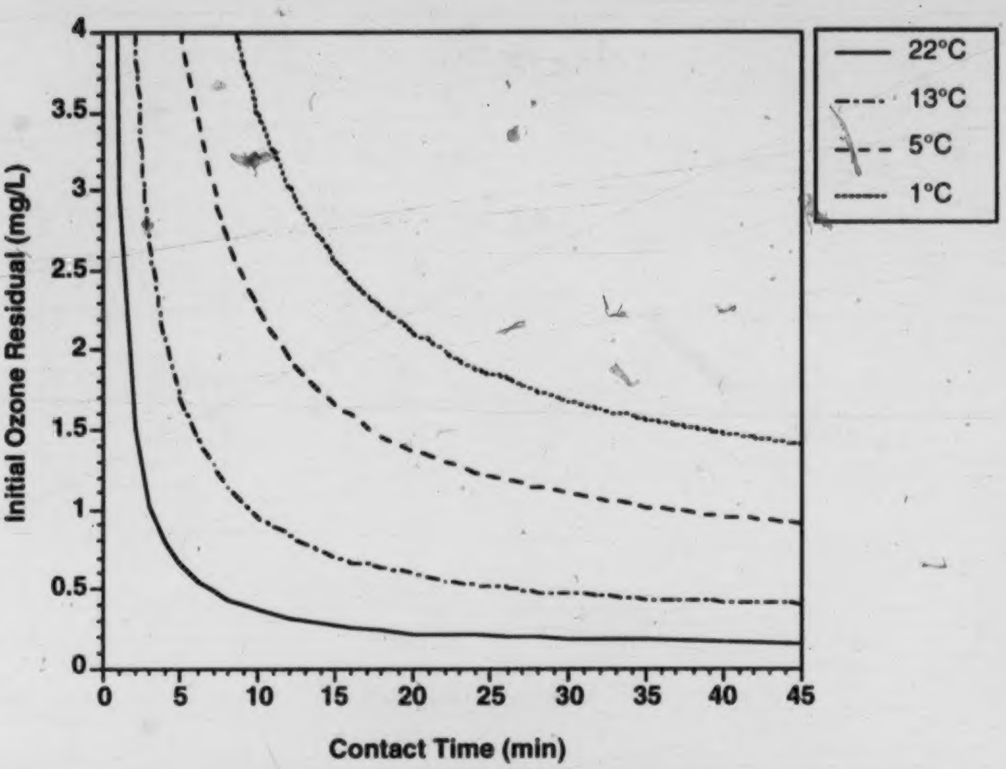


Figure 5-6. 1.0 log-unit design curves with 90% confidence level for ozone inactivation of *C. parvum* oocysts at pH 6 to 8 and 1 to 22°C given by I.g.H. modal, assuming first-order ozone disappearance rate constant of 0.05 1/min and buffered oxidant demand-free water

In application of the design criteria to natural waters, *in-situ* bench scale tests as a minimum are recommended to validate the model parameters for specific conditions. High alkalinity or organic content and other water quality parameters may have direct effects on ozone inactivation kinetics in addition to affecting ozone residual stability.

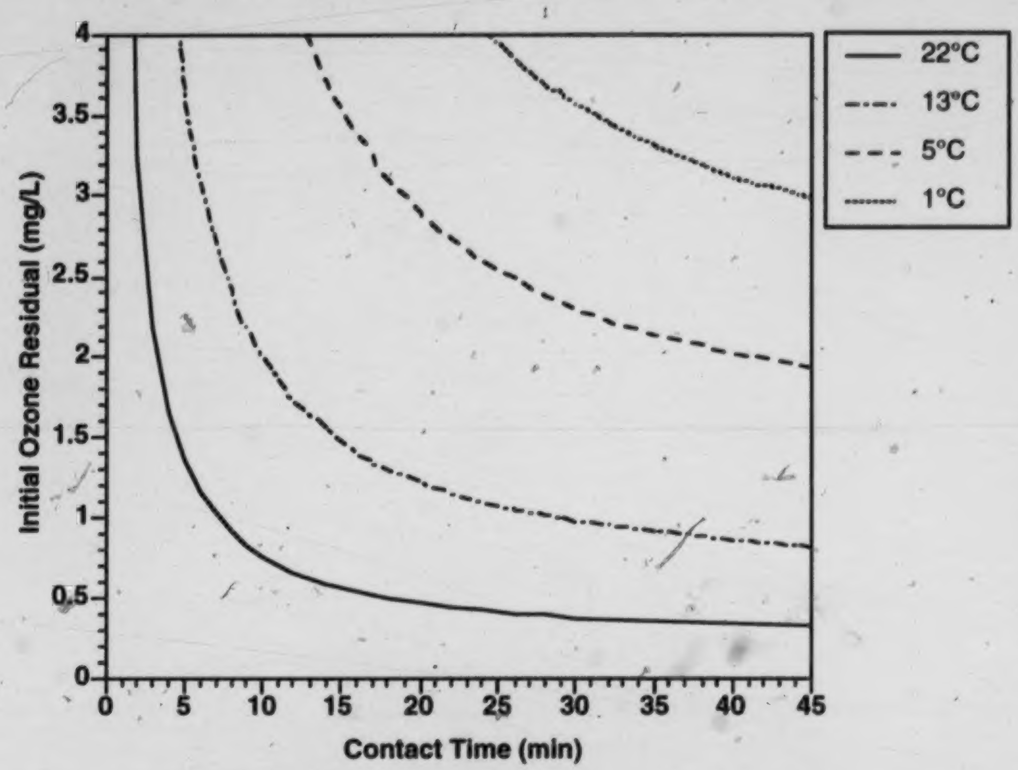


Figure 5-7. 2.0 log-units design curves with 90% confidence level for ozone inactivation of *C. parvum* oocysts at pH 6 to 8 and 1 to 22°C given by I.g.H. modal, assuming first-order ozone disappearance rate constant of 0.05 1/min and buffered oxidant demand-free water

5.5 SUMMARY

The kinetics of ozone inactivation of *C. parvum* oocysts were studied at 1 to 37°C, pH 6 to 8. Ozone was very effective in inactivating of *C. parvum* oocysts. A 3.0 log-unit inactivation could be achieved under the practical ozone dose and contact time at

22°C. Survival curves of ozone inactivation were characterized by an initial lag and an evident tailing. As temperature increased, the shoulder effect and the tail-off effect became less evident. The non-linear I.g.H. model gave an adequate fit for the disinfection kinetics.

Water temperature was critical for ozone inactivation of *C. parvum*, while pH was not a significant factor at pH 6 to 8. The effect of temperature on the reaction rate constant was predicted using the van't Hoff-Arrhenius relationship. For every 10°C increase, the I.g.H. model reaction rate constant increases by a factor of 2.2. The activation energy for ozone inactivation was estimated as 52 kJ/mol. Design criteria for 1.0 and 2.0 log-units of inactivation were developing during this study.

CHAPTER 6

CHLORINE DIOXIDE

6.1 INTRODUCTION

Chlorine dioxide was shown to be effective for control of *C. parvum* oocysts in water (Peeters et al. 1989; Korich et al. 1990b). When compared to ozone, chlorine dioxide treatment has the advantages of being more reaction selectivity, and its residual lasts longer in the water distribution systems (Aieta and Berg 1986). Preliminary studies of chlorine dioxide inactivation of *C. parvum* oocysts at low temperature showed that the chlorine dioxide efficacy decreased by about 50% for every 10°C reduction in temperature (Li and Finch 1998; Finch and Li 1999).

The goal of this portion of study was to explore the kinetics of *C. parvum* oocyst inactivation using chlorine dioxide at different pH and temperatures, based on the CD-1 mice infectivity assay. Kinetic models that describe the effect of water pH and temperature were developed. Design criteria using chlorine dioxide for control of *C. parvum* were developed.

6.2 EXPERIMENTAL SETTINGS

Experiment settings for applied dose and contact time followed the experimental design criteria described by Box and Lucas (1959). Considering the practical situation, the chlorine dioxide applied dose ranged from 0.4 to 6 mg/L with a contact time from 15 to 240 min. The inactivation level was targeted from zero to about 4 log-units. Higher doses and contact times were applied at low temperature to obtain the target inactivation.

Water pH and temperature were two major factors that were investigated. To examine the effect of water pH, paired tests were conducted at pH 6, 8 and 11 by applying the same doses and same contact times at the same temperature (1°C or 22°C). Assuming that the van't Hoff-Arrhenius theory was adequate for description of the temperature effect, data sets for two different temperatures, 1°C and 22°C, were collected to determine the empirical temperature coefficient constant, θ. Some data points were also collected at 5, 13 and 37°C to prove the fitness of van't Hoff-Arrhenius model.

The chlorine dioxide data were collected over a period of 3 years. Different batches of oocyst were used in the experiment. Consequently, the order of conducting the experiment at different temperature and pH was randomized so the data were independent from batches of oocysts used.

6.3 RESULTS AND DISCUSSION

6.3.1 Microbial Reduction Following Chorine Dioxide Treatment

Details of the experimental data for chlorine dioxide inactivation of *C. parvum* at room temperature (22°C) at pH 6, 8 and 11 are summarized in Table 6-1. Most trials

Table 6-1. Summary of chorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 11 and 22±1°C

Trial No.	pH	Initial ClO_2 residual C_o (mg/L)	Final ClO_2 residual C_f (mg/L)	Contact time t (min)	First order ClO_2 decay rate constant (1/min)	Reactor volume (mL))	Number of oocysts in reactor	Observed infectivity reduction (log-units)	C-W model predicted infectivity reduction (log-units)
577	6	1.91	1.86	15	2×10^{-3}	200	5.0×10^{7}	1.1	0.5
372	6	0.52	0.41	30	8×10^{-3}	200	1.0×10^{7}	-0.3	0.3
567	6	1.17	1.11	30	2×10^{-3}	200	5.0×10^{7}	0.4	0.6
556	[illegible]	3.98	3.84	30	1×10^{-3}	200	5.0×10^{7}	3.1	2.1
563	6	2.18	1.99	60	2×10^{-3}	200	2.8×10^{7}	2.7	2.3
640	6	0.96	0.85	61	2×10^{-3}	200	2.5×10^{7}	1.1	1.0
370	6	0.48	0.21	118	7×10^{-3}	200	1.0×10^{7}	0.3	0.7
566	6	1.16	1.01	120	1×10^{-3}	200	5.0×10^{7}	2.1	2.4
555	6	2.04	1.71	120	1×10^{-3}	200	5.0×10^{7}	3.7	4.1
265	8	0.39	0.25	30	10×10^{-3}	240	7.5×10^{6}	0.3	0.2
210	8	1.42	0.58	30	30×10^{-3}	25	3.5×10^{6}	1.0	0.5
270	8	0.58	0.41	41.5	50×10^{-3}	240	5.0×10^{6}	0.1	0.4
267	8	0.39	0.23	60	9×10^{-3}	240	7.5×10^{6}	0.2	0.3
564	8	2.1	1.94	60	1×10^{-3}	200	2.8×10^{7}	2.6	2.2
218	8	2.91	0.65	60	20×10^{-3}	25	3.5×10^{6}	2.1	1.6
268	8	0.98	0.54	120	5×10^{-3}	240	7.5×10^{6}	0.9	1.6
572	8	1.19	0.84	120	3×10^{-3}	200	5.0×10^{7}	1.9	2.2
598	11	1.86	1.76	15	4×10^{-3}	200	5.0×10^{7}	0.6	0.5
376	11	0.41	0.12	30	40×10^{-3}	200	1.0×10^{7}	0.0	0.1
382	11	3.98	3.24	30	7×10^{-3}	200	1.0×10^{7}	2.0	2.0
597	11	1.99	1.36	60	6×10^{-3}	200	5.0×10^{7}	1.5	1.8
638	11	0.94	0.6	61	7×10^{-3}	200	2.5×10^{7}	1.4	0.8
374	11	1.99	0.94	90	8×10^{-3}	200	1.0×10^{7}	2.0	2.3
573	11	1.1	0.57	120	5×10^{-3}	200	5.0×10^{7}	1.6	1.8
380	11	2.05	0.5	120	10×10^{-3}	200	1.0×10^{7}	2.5	2.4
373	11	3.43	0.93	120	10×10^{-3}	200	4.0×10^{7}	3.3	4.2

* Model is expressed as Equation 3-11 and parameters are listed in Table 6-4

were conducted using the 250 mL reactor with the buffer volume of 200 mL. Initial concentration of the chlorine dioxide ranged from 0.39 to 3.98 mg/L and the contact time ranged from 15 to 120 min. The observed kills were up to 3.7 log-units. In some trials using low doses of chlorine dioxide, negative kills were observed due to the variability of the infectivity assay. Typical chlorine dioxide first-order decay observed at room temperature ranged from 0.001 to 0.01 1/min.

The low temperature (1°C) data at pH 6 and 8 are summarized in Table 6-2. At 1°C, much higher Ct products were necessary to achieve the same level of kill as at 22°C. The initial chlorine dioxide concentration used for the low temperature experiments ranged from 1.0 to 6.05 mg/L and the contact time ranged from 30 to 240 min. The observed kills ranged from 0.1 to 2.9 log-units. A pseudo-replicate (*i.e.* Trial Nos. 528 and 507) showed that an initial concentration of 4.48 to 4.61 mg/L for 120 min ($C_{avg}t$ about 546 mg·min/L) at pH 6 resulted in an inactivation of 2.0 to 2.3 log-units. A chlorine dioxide dose of 4.5 mg·min/L for 180 min ($C_{avg}t$ product about 810 mg·min/L) resulted in the inactivation of 2.5, 2.8 and 2.8 log-units for pH 6, 8 and 11, respectively (*i.e.* Trial Nos. 617, 605 and 606). Chlorine dioxide decay at 1°C was between 0.0001 to 0.003 1/min, which were lower than those at room temperature.

Inactivation data were also collected at pH 6 and 5, 13 and 37°C (Table 6-3). The results clearly demonstrated the temperature effect on chlorine dioxide inactivation of *C. parvum* oocysts. At 5°C, $C_{avg}t$ product of about 480 mg·min/L (initial chlorine

Table 6-2. Summary of chorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 11 and 1±0.5°C

Trial No.	pH	Initial ClO_2 residual C_o (mg/L)	Final ClO_2 residual C_f (mg/L)	Contact time t (min)	First order ClO_2 decay rate constant (1/min)	Reactor volume (mL)	Number of oocysts in reactor	Observed infectivity reduction (log-units)	C-W model predicted infectivity reduction* (log-units)
494	6	4.65	4.51	30	1×10^{-3}	200	2.5×10^{7}	0.5	0.4
500	6	1.16	1.11	60	0.7×10^{-3}	200	5×10^{7}	1.0	0.2
506	6	1.20	1.11	60	1×10^{-3}	200	5×10^{7}	0.5	0.2
528	6	4.48	4.17	120	0.6×10^{-3}	200	5×10^{7}	2.3	1.7
507	6	4.61	3.27	120	2×10^{-3}	200	5×10^{7}	2.0	1.5
498	6	2.27	2.08	180	0.5×10^{-3}	200	5×10^{7}	1.2	1.3
617	6	4.71	3.43	180	2×10^{-3}	500	7.5×10^{7}	2.5	2.4
627.1	6	2.04	1.90	230	0.3×10^{-3}	500	7.5×10^{7}	1.6	1.5
496	6	1.00	0.88	240	0.5×10^{-3}	200	2.5×10^{7}	0.0	0.7
596	6	2.20	1.45	240	2×10^{-3}	200	5×10^{7}	1.1	1.4
497	6	4.50	4.18	240	0.3×10^{-3}	200	2.5×10^{7}	2.9	3.4
667	8	4.51	4.46	30	0.4×10^{-3}	200	1×10^{7}	0.3	0.4
586	8	1.24	1.08	120	1×10^{-3}	200	5.0×10^{7}	1.0	0.5
605	8	4.33	3.89	180	0.6×10^{-3}	200	2.5×10^{7}	2.8	2.4
624.1	8	1.92	1.78	235	0.3×10^{-3}	500	7.5×10^{7}	1.4	1.4
662	11	4.60	4.40	30	1×10^{-3}	200	1.0×10^{7}	0.4	0.4
668	11	1.28	1.06	120	2×10^{-3}	200	1.0×10^{7}	0.1	0.5
588	11	1.41	1.00	120	3×10^{-3}	200	2.5×10^{7}	0.0	0.5
584	11	6.05	5.10	120	1×10^{-3}	200	5.0×10^{7}	1.9	2.2
606	11	4.33	3.89	180	0.6×10^{-3}	200	2.5×10^{7}	2.8	2.4
618	11	4.55	3.65	180	1×10^{-3}	500	7.5×10^{7}	2.6	2.4
663	11	1.82	1.61	240	0.5×10^{-3}	200	1.0×10^{7}	1.0	1.3

* Model is expressed as Equation 3-11 and parameters are listed in Table 6-4

Table 6-3. Summary of chlorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 5, 13 and 37 °C

Trial No.	Temp. T (°C)	Initial ClO_2 residual C_o (mg/L)	Final ClO_2 residual C_f (mg/L)	Contact time t (min)	First order ClO_2 decay rate constant (1/min)	Number of oocysts in reactor	Observed infectivity reduction (log-units)	C-W model predicted infectivity reduction* (log-units)
439	5	0.57	0.48	30	6×10^{-3}	1.0×10^{7}	-0.1	0.1
609	5	2.10	2.00	60	0.8×10^{-3}	2.5×10^{7}	0.1	0.6
587	5	2.11	2.00	60	0.9×10^{-3}	2.5×10^{7}	0.2	0.6
608	5	4.22	4.1	60	0.5×10^{-3}	2.5×10^{7}	1.1	1.1
448	5	4.77	4.23	60	2×10^{-3}	1.0×10^{7}	1.1	1.2
437	5	1.54	1.13	120	3×10^{-3}	1.0×10^{7}	1.3	0.7
529	5	4.12	3.88	120	0.5×10^{-3}	5.0×10^{7}	2.8	2.2
446	5	4.74	3.90	120	2×10^{-3}	1.0×10^{7}	1.8	2.3
542	13	2.46	2.39	30	1×10^{-3}	5.0×10^{7}	0.9	0.6
576	13	1.94	1.87	60	0.6×10^{-3}	5.0×10^{7}	1.0	1.0
541	13	1.95	1.75	150	0.7×10^{-3}	5.0×10^{7}	2.9	2.4
600	37	2.00	1.66	16	10×10^{-3}	5.0×10^{7}	2.3	1.8
599	37	1.02	0.93	30	0.3×10^{-3}	5.0×10^{7}	2.2	1.8

* Model is expressed as Equation 3-11 and parameters are listed in Table 6-4;
Reactor volume of all the trials was 200 mL.

dioxide of 4.12 to 4.74 for 120 min) caused an inactivation of 1.8 to 2.8 log-units. In contrast, a $C_{avg}t$ product of 30 mg·min/L at 37°C (chlorine dioxide dose of 1.02 mg/L for 30 min) caused an inactivation of 2.2 log-units. Thus, the required chlorine dioxide $C_{avg}t$ values for a similar inactivation increased dramatically with the decrease in temperature.

6.3.2 Inactivation Kinetics

Survival ratios *versus* the chlorine dioxide $C_{avg}t$ at temperatures of 1 to 37 °C were plotted in Figure 6-1. Survival curves of *C. parvum* oocysts exposed to chlorine dioxide at different temperatures declined linearly with the rise in chlorine dioxide $C_{avg}t$ product. The absolute value of the slope of the survival curves increased dramatically at

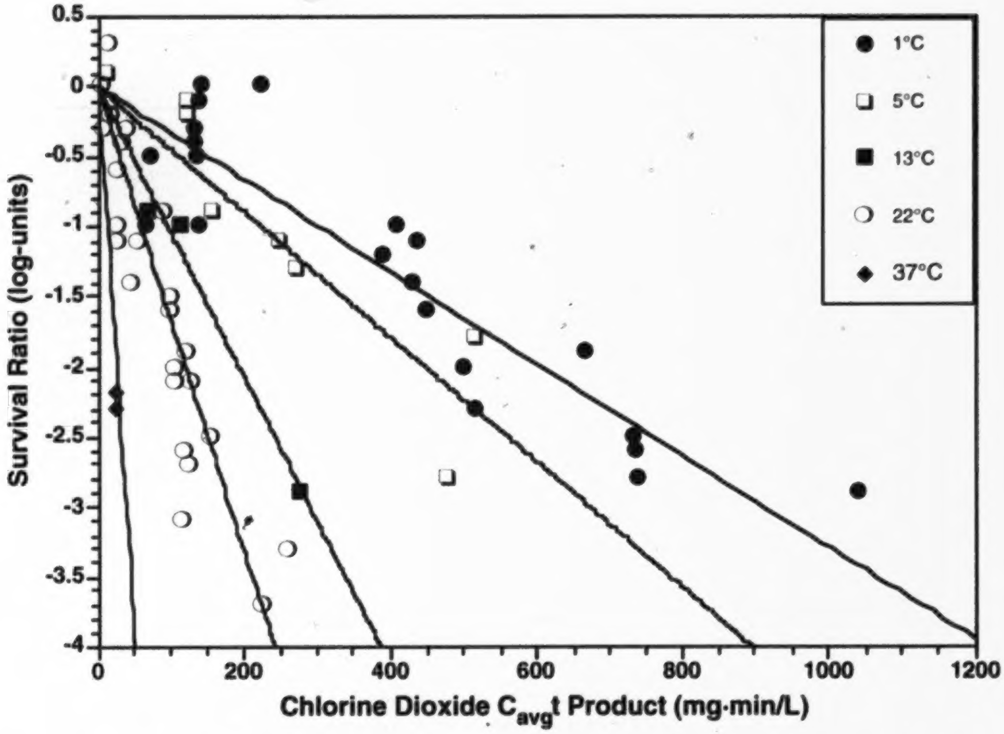


Figure 6-1. Survival ratios of *C. parvum* oocysts as the function of $C_{avg}t$ products after chlorine dioxide treatment in oxidant demand-free 0.05 M phosphate buffer at temperature from 1 to 37°C (the lines across the origin are the best fit curves at different temperatures)

high temperature. The data at pH 6, 8 and 11 at the same temperature (1 or 22°C) were indistinguishable in the figure, suggesting that water pH might not be a significant factor for chlorine dioxide inactivation.

The linear Chick-Watson model (Equation 3-5) was calibrated for chlorine dioxide inactivation at 1 or 22°C. First order disinfectant decay was accounted for in the model since the chlorine dioxide decay varied from trial to trial. Model parameters and their marginal intervals with 90% confidence level are summarized in Table 6-4. The reaction rate constant, k, at 22°C was about 5 times that at 1°C ($\hat{k}_{22}$=0.017 L/(mg·min) and $\hat{k}_1$=0.0033 L/(mg·min)). A single Chick-Watson model with temperature correction (Equation 3-11) was estimated for the temperature range from 1 to 37°C and pH from 6 to 11. The Chick-Watson model with temperature correction based on van't Hoff-Arrhenius relationship gave a very good prediction at a temperature range from 1 to 37 (Figure 6-2a). The model variance given by the data was σ=0.41 log-units with 59 degrees of freedom. Assuming sample normality, the 90% confidence intervals for the model prediction was $\pm Z(1-\alpha/2)\times\sigma = \pm 0.6$ log-units.

A temperature corrected I.g.H. model (Equation 3-13) was also derived using the pooled data for temperatures ranging from 1 to 37°C and pH 6 to 11 (Table 6-4). The n and m value of the I.g.H. model parameters were very close to unity ($\hat{m}$= 0.78 and $\hat{n}$ = 1.00). Standard deviation of the I.g.H. model was σ=0.36, which was slightly lower than that of the Chick-Watson model. From zero to 3.0 log-units of inactivation, the I.g.H.

model basically gave the same prediction as the Chick-Watson model. Thus, the much simpler linear Chick-Watson model was recommended for the prediction of chlorine dioxide inactivation of *C. parvum* oocysts in water.

Table 6-4. Model parameters for chlorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 8 and 1 to 37°C.

Kinetic model	**Chick-Watson**[*]				**I.g.H.**[†]
Temperature (°C)	1	22	1 to 37	1 to 37	1 to 37
pH	6, 8, 11	6, 8, 11	6	6, 8, 11	6, 8, 11
Number of trials	20	24	30	61	61
Temperature correction, $\hat{\theta}$ (±90% limits)	-	-	1.09 (1.08, 1.09)	1.085 (1.08, 1.09)	1.08 (1.07, 1.08)
$\hat{k}$ (±90% limits)	0.0033 (1°C) (0.003, 0.004)	0.017 (22°) (0.016, 0.018)	0.020 (22°C) (0.018, 0.021)	0.018 (22°C) (0.017,0.019)	0.039 (22°C) (0.37, 0.41)
$\hat{m}$ (±90% limits)					0.81 (0.80, 0.82)
$\hat{n}$ (±90% limits)					1.07 (1.02, 1.11)
error, σ	0.39	0.42	0.35	0.41	0.36
Model constraints: Initial residual C_o (mg/L) Contact time t (min)	$0.88 \le C_o \le 6.05$ $30 \le t \le 240$	$0.21 \le C_o \le 3.98$ $15 \le t \le 120$	$0.21 \le C_o \le 4.71$ $15 \le t \le 240$	$0.21 \le C_o \le 6.05$ $15 \le t \le 240$	$0.21 \le C_o \le 6.05$ $15 \le t \le 240$

* Equation 3-5 for single temperature and Equation 3-11 for multiple temperatures;
† Equation 3-13.

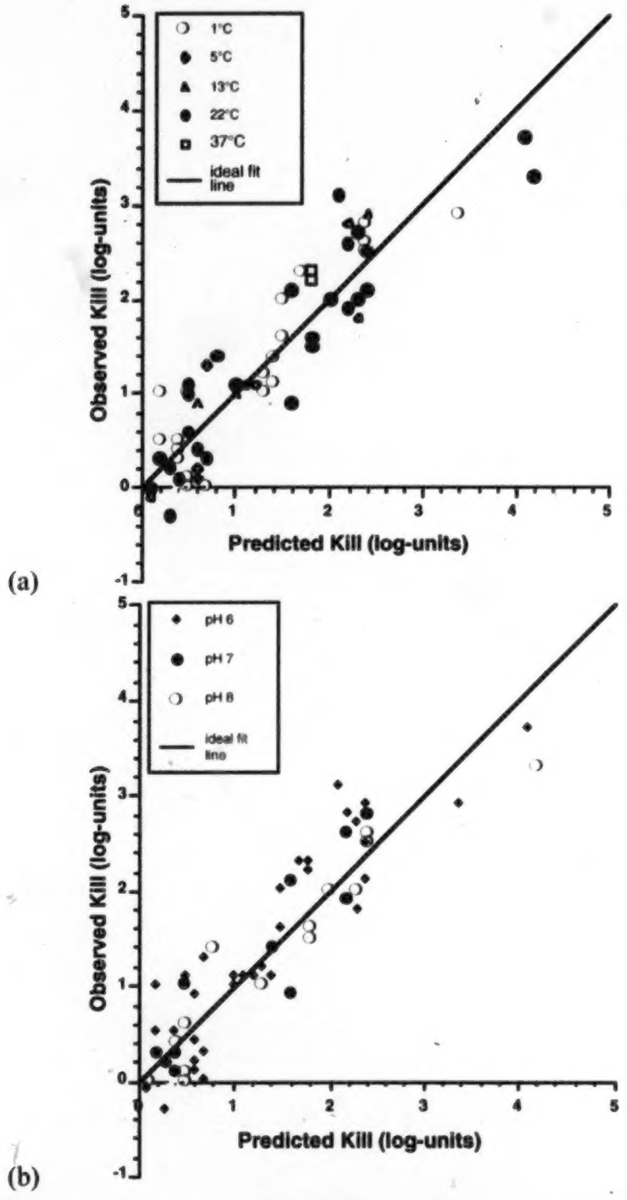


Figure 6-2. Temperature corrected Chick-Watson model fit for chlorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 to 11 and 1 to 37°C: a) as a function of temperature, and b) as a function of pH

Non-linear I.g.H. model was used for ozone inactivation of *C. parvum* oocysts (Finch et al. 1994b; Liyanage et al. 1997b; Gyürék et al. 1999) due to the nonlinear nature of the *C. parvum* oocyst survival curses under ozone treatment. The temperature corrected I.g.H. model gave a good fit for the shoulder and tailing-off effect in the survival curves. For chlorine dioxide treatment, the use of I.g.H. type model would be unnecessary although slight shoulder and tailing-off did exist in the survival curves. For chlorine dioxide inactivation of *C. parvum* oocysts at temperature 1 to 37°C and pH 6 to 11, the variance-convariance matrice (Equation 3-32) of the four-parameter ($\hat{\theta}$, $\hat{k}_{22}$, $\hat{m}$, $\hat{n}$) temperature corrected I.g.H. model was estimated as

$$\hat{\Sigma} = \begin{bmatrix} 3.62\text{E}-5 & -5.90\text{E}-5 & 3.27\text{E}-4 & 3.59\text{E}-4 \\ & 1.46\text{E}-4 & -8.14\text{E}-4 & -5.84\text{E}-4 \\ & & 4.68\text{E}-3 & 2.68\text{E}-3 \\ & & & 6.34\text{E}-3 \end{bmatrix}$$

from which the correlation matrice was

$$\text{R} = \begin{bmatrix} 1 & -0.81 & 0.79 & 0.75 \\ & 1 & -0.98 & -0.61 \\ & & 1 & 0.49 \\ & & & 1 \end{bmatrix}$$

In the temperature corrected I.g.H. model (Table 6-4), $\hat{m}=0.81$ and $\hat{n}=1.07$ were estimated from the data. The correlation factor between $\hat{k}_{22}$ and $\hat{m}$ was -0.98 in the correlation matrice, suggesting these two parameters were highly confounded to each other. Thus, it was feasible to simplify and model by assuming m = 1 so both m and n were unity, resulting in the standard Chick-Watson type kinetic model (Equation 3-11).

6.3.3 pH Effect

From the paired tests of chlorine dioxide inactivation at different pH values, similar kills at pH 6 to 11 were observed when the same dose and contact time were used. For example, at 1°C about 4.5 mg/L initial chlorine dioxide for 30 min resulted in 0.5, 0.3 and 0.4 log-units inactivation at pH 6, 8 and 11, respectively (Table 6-2). About 4.5 mg/L initial chlorine dioxide for 180 min at 1°C, resulted in 2.5, 2.8 and 2.6 log-units inactivation at pH 6, 8 and 11, respectively. Other data at 1 and 22°C for different pH values (Table 6-1 and Table 6-2) supported a similar conclusion. The results indicated that the inactivation differences at variable pH values were within the variance of animal infectivity assay. The fitness plot of the Chick-Watson model at pH 6 to 11 (Figure 6-2b) also showed that a single model could be used for prediction of chlorine dioxide inactivation at pH 6 to 11. The temperature corrected Chick-Watson model for pH 6 to 11 was similar to the model for the pH 6 alone (Table 6-4). Standard deviation of the two models were similar (s =0.41 for pH 6 to11 and s =0.35 for pH 6 alone).

Bernarde et al. (1965) reported that chlorine dioxide was more effective at pH 8.5 than at pH 6.5 for inactivation of *Escherichia coli*. Chen et al. (1985) reported that chlorine dioxide inactivation of *Neagleria gruberi* cysts at pH 9 was significantly higher than at pH 5 to 7. Noss and Olivieri (1985) studied the chlorine dioxide inactivation of bacterial virus f2 under acidic, neutral and alkaline conditions, and reported that the rate of inactivation increased with pH. Interestingly, Junli et al. (1997) reported a decrease of chlorine dioxide efficacy for Poliovirus I inactivation and credited the pH effect to the degradation of chlorine dioxide into chlorate ion and chlorite ion. It seems the effect of pH on chlorine dioxide inactivation may vary among different microorganisms and depends on the test conditions.

Our studies on ozone inactivation of *C. parvum* already showed that the reaction rate constant was independent of water pH at pH 6 to 8 (Gyürék et al. 1999). The residual chlorine dioxide was relatively stable over a wide pH range, although at high pH it degraded into chlorate ion and chlorite ion more readily (Bryant et al. 1992). Assuming that chlorine dioxide was the active component in *C. parvum* inactivation (Liyanage et al. 1997a), the chlorine dioxide decay should not affect the reaction rate constant in the kinetic models. Study of the surface properties of the oocysts (Drozd and Schwartzbrod 1996) showed that a higher negative surface charge was apparent at higher pH. It remains to be determined whether change in the surface charge of the oocysts affects their susceptibility to chlorine dioxide inactivation.

6.3.4 Temperature Effect

Water temperature was a critical factor for chlorine dioxide inactivation of *C. parvum* oocysts. Based on the temperature corrected Chick-Watson model, the temperature coefficient, $\hat{\theta}=1.085$, was given by the data from 1 to 37°C, pH 6 to 11. For every 10°C temperature increase, the reaction constant increased about 2.3 times. An estimate of temperature coefficient using the nonlinear I.g.H. model was $\hat{\theta}=1.08$, which was statistically the same as that given by the Chick-Watson model.

The US EPA suggested a temperature coefficient value for *Giardia* spp. cysts inactivation was 1.07 (Malcolm Pirnie Inc. and HDR Engineering Inc. 1991), which is similar to that for *C. parvum* oocysts obtained in this study. The activation energy (E_a) for chlorine dioxide inactivation of *C. parvum* oocysts was estimated to be 55 kJ/mol and the pre-exponential factor (A) was 1.0×10^8 1/min. Published activation energies of chlorine dioxide inactivation of *N. gruberi*, Poliovirus-1 and *E. coli* are shown in Table 6-5. The activation energy of chlorine dioxide for *C. parvum* was similar to that of *E. coli* (Benarde et al. 1967), but was lower than that for *N. gruberi* cysts (Chen et al. 1985). Ruffell et al. (2000) used *in vitro* excystation to study chlorine dioxide inactivation of

Table 6-5. Activation energies for chlorine dioxide inactivation of microorganisms

Microorganisms	E_a (kJ/mol)	pH	Temperature (°C)	Reference
C. parvum oocysts *	55	6 to 8	1 to 37	This study
C. parvum oocysts †	86.3	8	4 to 30	(Ruffell et al. 2000)
Naegleria gruberi cysts	80.2 to 85.2	5 to 9	5 to 30	(Chen et al. 1985)
Poliovirus-1	35.1	7	15 to 25	(Junli et al. 1997)
Escherichia coli	50.2	6.5	5 to 32	(Benarde et al. 1967)

* Based on animal infectivity and Chick-Watson model;
† Based on *in vitro* excystation and pseudo-first order model.

C. parvum and reported an activation energy of 86.3 kJ/mol, which was more than 50% higher than the one obtained in this study using animal infectivity assay.

6.3.5 Chlorine Dioxide Disinfection Design Criteria

Chlorine dioxide inactivation of *C. parvum* was predicted using the temperature corrected Chick-Watson model. A Ct table for 0.5 to 2.0 log-units inactivation at 1 to 22°C given by the temperature corrected model is shown in Table 6-6. At room temperature, the $C_{avg}t$ values estimated for an inactivation of 1.0 and 2.0 log-units were about 56 and 111 mg·min/L, respectively.

Table 6-6. Ct requirement for chlorine dioxide inactivation of *C. parvum* oocysts at pH 6 to 11 given by the Chick-Watson model, assuming constant chlorine dioxide residual in oxidant demand-free 0.05 M phosphate buffer (no safety factor)

Temperature	1°C	5°C	13°C	22°C
Inactivation level (log-units)	Chlorine dioxide Ct product (mg·min/L)			
0.5	152	110	51	28
1.0	303	221	115	56
1.5	455	331	172	83
2.0	606	442	230	111

Peeters et al. (1989) reported that one log-unit of inactivation required a Ct value of 6 to 13 mg·min/L at room temperature and neutral pH. Korich et al. (1990b) reported that an chlorine dioxide Ct of 59 to 78 mg·min/L was required for 2 log-units of inactivation at pH 7 at 25°C. The required Ct values obtained in this study for same levels of inactivation are higher than those given by Korich et al. (1990b) and Peeters et al. (1989).

The chlorine dioxide contactor designed with the model predict $C_{avg}t$ without a safety factor, and only gives a 50% assurance for the target inactivation levels. To obtain a higher confidence levels, a simple approach would be to add a safety factor to the theoretical $C_{avg}t$. However a more reliable approach would be to calculate design $C_{avg}t$ with a certain confidence level based on the data used in model parameter estimation. Design curves targeting 0.5, 1.0 and 2.0 log-units inactivation with 90% confidence level at different temperatures were developed in this study based on the Chick-Watson model (Figure 6-3, Figure 6-4 and Figure 6-5). A first-order chlorine dioxide disappearance rate constant of 0.001 1/min was assumed in the calculation. The design criteria to ensure a 90% confidence level is approximately equivalent to a safety factor of 1.7 on the theoretical dose for that target inactivation. The contact time, t, is the detention time in the ideal plug flow reactor. For a non-ideal plug flow reactor, t_{10}, the minimum exposure time for 90% of the reactor content, can be used as the design contact time in a standard approach.

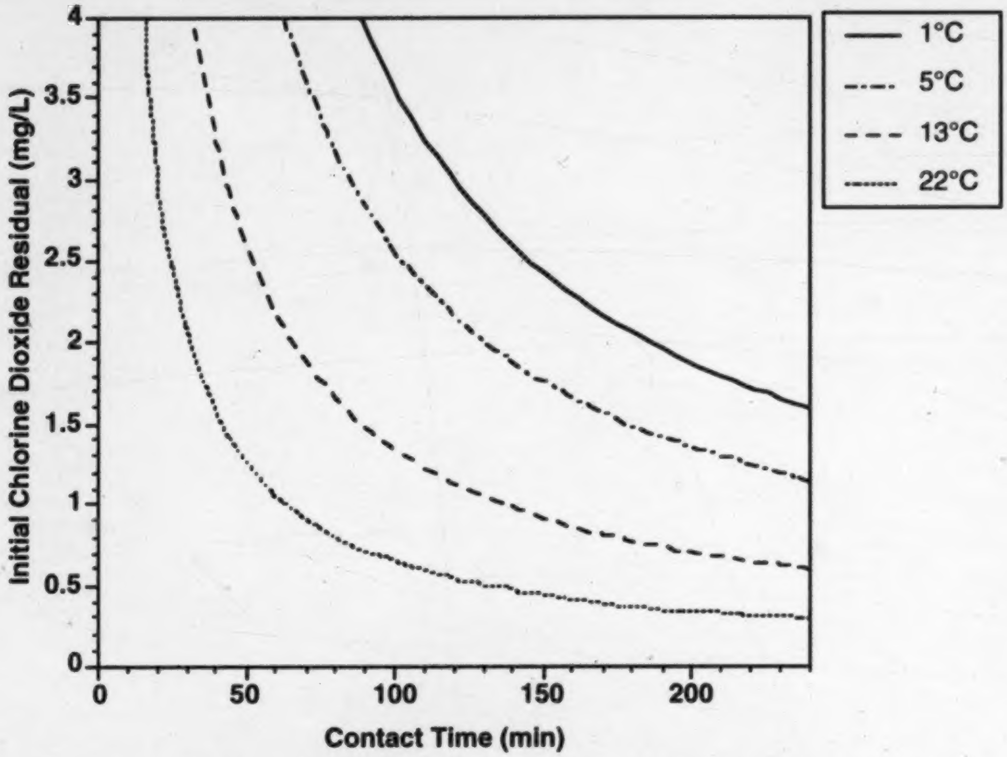


Figure 6-3. 0.5 log-units design curves with 90% confidence level for chlorine dioxide inactivation of *C. parvum* oocysts at pH 6 to 11 and 1 to 22°C given by Chick-Watson model, assuming first-order chlorine dioxide disappearance rate constant of 0.001 1/min (using oxidant demand-free 0.05 M phosphate buffer)

One big concern with adding chlorine dioxide to water was the toxicity of chlorine dioxide, chlorite ion and chlorate ion (Lykins et al. 1990). Because a total chlorine dioxide residual oxidants of 0.8 mg/L is recommended by the US EPA (1998a),

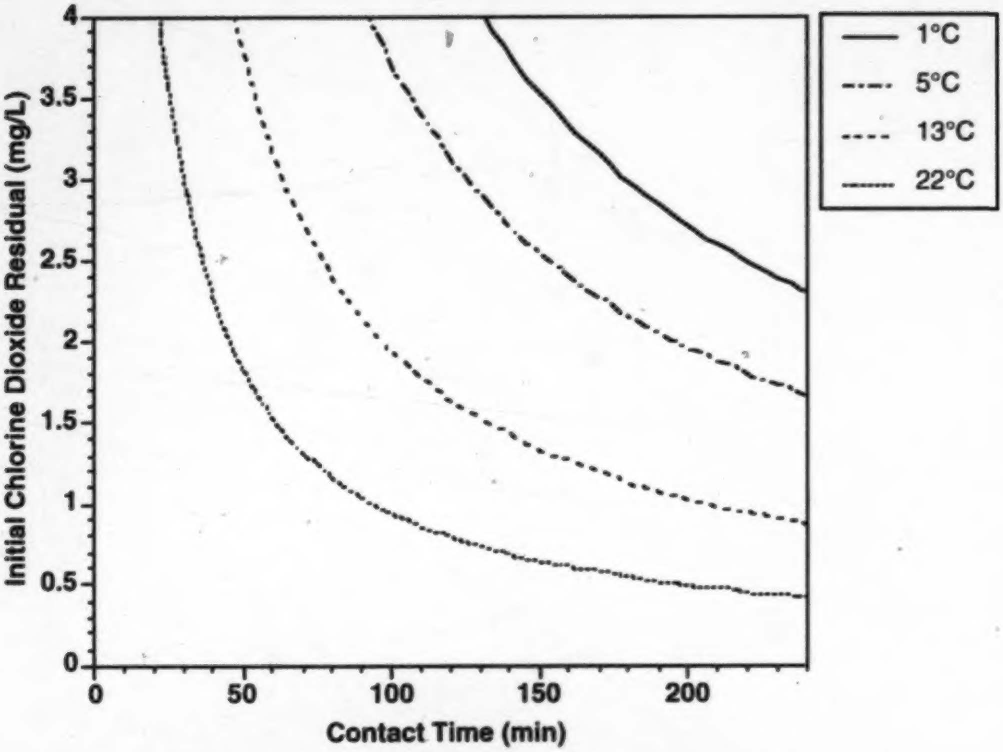


Figure 6-4. 1.0 log-unit design curves with 90% confidence level for chlorine dioxide inactivation of *C. parvum* oocysts at pH 6 to 11 and 1 to 22°C given by Chick-Watson model, assuming first-order chlorine dioxide disappearance rate constant of 0.001 1/min (using oxidant demand-free 0.05 M phosphate buffer)

the highest practical chlorine dioxide dose is about 1.1 mg/L. Thus, the inactivation level achievable by chlorine dioxide was limited by the practical dose limit. From the 90% confidence design curves (Figure 6-3, Figure 6-4 and Figure 6-5),

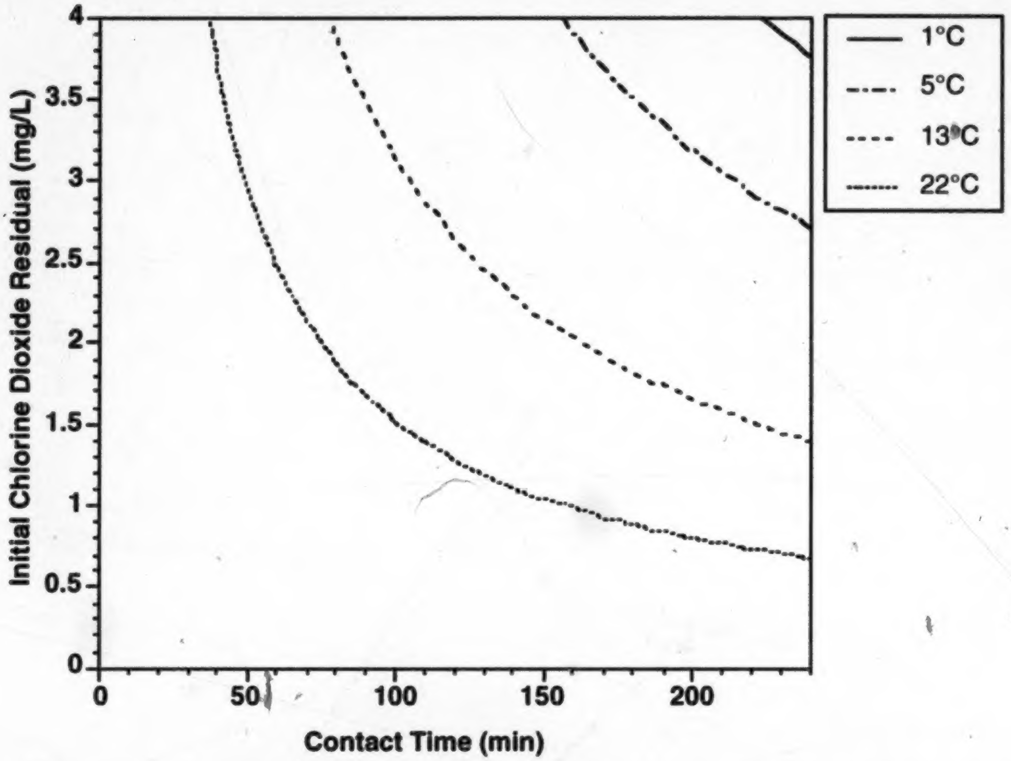


Figure 6-5. 2.0 log-units design curves with 90% confidence level for chlorine dioxide inactivation of *C. parvum* oocysts at pH 6 to 11 and 1 to 22°C given by Chick-Watson model, assuming first-order chlorine dioxide disappearance rate constant of 0.001 1/min (using oxidant demand-free 0.05 M phosphate buffer)

a contactor with a detention time of 120 min might be able to achieve 2.0 log-units inactivation when 1.1 mg/L chlorine dioxide is applied. However, in most cities in North America low temperature water in winter or spring imposes a challenge for chlorine dioxide treatment processes. For the same chlorine dioxide $C_{avg}t$ product the inactivation efficacy decreased about 2.3 times for every 10°C. Assuming the contact time can be up to 120 min, it may be feasible to achieve 1 log-unit inactivation above 13°C. However, at 5°C, only a 0.5 log-units of inactivation was achievable for the same contact time. The drawback of low temperature might be compensated by a higher chlorine dioxide dose or a longer contact time. Other approaches such as sequential disinfection might help improve the chlorine dioxide performance at low temperature (Liyanage et al. 1997b; 1997d). In the natural waters where chlorine dioxide demand is high, more chlorine dioxide will be needed to achieve the target initial concentration. Consequently, more chlorite ion and chlorate ion will be formed in the water. Disinfection by-product removal should be considered if the concentration exceeds the limits, however, adding a chlorite ion and chlorate ion removal unit will increase the cost.

6.4 SUMMARY

The kinetics of chlorine dioxide inactivation of *C. parvum* oocysts in oxidant demand-free phosphate buffer were studied at temperature 1 to 37°C and pH 6 to 11. Survival curves of *C. parvum* oocysts under chlorine dioxide inactivation declined approximately linearly with the chlorine dioxide $C_{avg}t$ product. Water temperature was a critical factor for chlorine dioxide inactivation while water pH was not statistically important to the reaction rate constant.

The Chick-Watson model was suitable for prediction of chlorine dioxide inactivation. Within the experimental temperatures, van't Hoff-Arrhenius theory was adequate in estimating the change of the reaction rate constant with water temperature. The reaction rate constant of kinetic model decreased by a factor of 2.3 for every 10°C decrease at water temperature, corresponding to the activation energy of 55 kJ/mol.

Design criteria for chlorine dioxide inactivation of *C. parvum* targeting 0.5, 1.0 and 2.0 log inactivation with 90% confidence level were developed for different temperatures. The result showed that it may be feasible to achieve 2.0 log-units inactivation of *C. parvum* oocysts at room temperature and about 1.0 log-units inactivation at 13°C, given the contact time of 150 min.

CHAPTER 7

CHLORINE SPECIES

7.1 INTRODUCTION

Free chlorine and monochloramine are the most widely used microbial reduction chemicals in water treatment. At pH 6 and temperature 20°C, about 97% of the free chlorine exits as the form of hypochlorous acid, and at lower temperatures the percentage of hypochlorous acid is even higher (Sawyer and McCarty 1978). Hypochlorous acid has been shown to be more effective than hypochlorite ion in microorganism reduction. Chloramine forms when ammonium presents in water during chlorination. Monochloramine is often applied as the secondary chemical to maintain a residual in water distribution system.

The kinetic models for free chlorine and monochloramine inactivation *of C. parvum* oocysts at room temperature were developed by Gyürék et al. (1997). However, data for low temperatures were limited and the performance of free chlorine and monochloramine at low temperatures was not well defined. The objective of this portion of study was to examine the temperature effect on free chlorine or monochloramine inactivation.

7.2 EXPERIMENTAL SETTINGS

Experiments for free chlorine were conducted at pH 6 in oxidant demand free 0.05 M phosphate buffer. To develop a temperature corrected model, data were collected at 1, 10 and 22°C using different free chlorine doses and contact times. Chlorine doses for most trials were 1 to 4 mg/L, and contact times were lower than 990 min.

The monochloramine experiments were conducted at pH 8 in oxidant demand-free 0.05 M phosphate buffer. Oxidant was added as preformed monochloramine, prepared by mixing chlorine with ammonia at the Cl_2 : NH_3 –N mass ratio of 3 :1 at pH 8. Experiments were also conducted at 1, 10 and 22°C with different doses and contact times to develop the kinetic model for different temperatures. To meet the practical dose limit for drinking water treatment, the majority of the monochloramine doses were less than 5 mg/L, and contact times were less than 980 min.

7.3 RESULTS WITH FREE CHLORINE

7.3.1 Microorganism Reduction After Free Chlorine Treatment

The results of free chlorine inactivation of *C. parvum* oocysts at pH 6 at 1 and 10°C are summarized in Table 7-1. At 1°C, the free chlorine doses less than 5.4 mg/L with a contact time ranging from 240 to 990 min resulted in an inactivation of less than 0.6 log-units. Triplicate tests with an initial free chlorine dose of 3 mg/L and contact

Table 7-1. Summary of free chlorine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1 to 22 °C

Trial No.	Temp. T (°C)	Initial Cl_2 residual C_o (mg/L)	Final Cl_2 residual C_f (mg/L)	Contact time t (min)	Reactor volume (mL)	Number of oocysts in reactor	Observed Infectivity reduction (log-units)	Predicted infectivity reduction (log-units)
631.3	1	1.1	0.91	240	400	5×10^7	0.3	0.0
746.1	1	3.0	3.0	240	200	5×10^6	0.4	0.0
627.3	1	3.8	3.8	240	400	5×10^7	0.3	0.1
746.2	1	3.0	3.0	480	200	5×10^6	0.2	0.1
746.3	1	3.0	2.9	960	200	5×10^6	0.3	0.2
826.2	1	3.0	2.8	960	200	5×10^7	-0.1	0.2
656	1	3.3	3	960	200	5×10^6	0.2	0.2
627.5	1	3.8	3.8	960	400	5×10^7	0.6	0.2
776.2	1	5.4	4.9	960	200	5×10^6	0.1	0.3
753.1	10	3.0	2.9	480	200	5×10^6	0.1	0.2
753.2	10	3.0	2.8	960	200	5×10^6	0.3	0.4
824.1	10	1.1	0.8	990	200	5×10^6	-0.3	0.1
823.1	10	3.9	3.5	990	200	5×10^6	0.4	0.5
*35**	22	13.7	13.7	60	50	1×10^7	0.1	0.3
*141**	22	14.2	14.1	160	25	5×10^6	1.2	0.7
*37**	22	3.8	3.8	240	50	1×10^6	0.1	0.3
*102**	22	11.0	11	240	25	5×10^6	0.6	0.8
*74**	22	3.9	3.8	245	25	5×10^6	0.6	0.3
*249**	22	1.73	1.6	1030	475	7.5×10^6	0.3	0.5

*Source: (Gyürék et al. 1997), control adjustment was not applied in reduction of infectivity.

time of 960 min at 1°C resulted in a kill from -0.1 to 0.3 log-units. At 10°C, an initial free chlorine dose of 3.9 mg/L and contact time of 990 min resulted in an inactivation of 0.4 log-units. Under the practical free chlorine dose and contact time, little inactivation was observed for free chlorine.

To develop a temperature corrected kinetic model for free chlorine inactivation of *C. parvum* oocysts, the room temperature (22°C) data collected using similar protocols

(Gyürék et al. 1997) were re-evaluated and combined with the low temperature data. Censored data and some data with an extremely high oxidant doses were not included in the analysis since they were beyond the interest of water treatment practice. As discussed before, the observed kill in Table 7-1 was not adjusted by the kill of individual control since the expected kill from the control trials was close to zero. Under the practical dose and contact time, less than one log-unit of inactivation was observed following treatment using free chlorine at room temperature.

7.3.2 Kinetic Modeling

Chick-Watson model with temperature correction was calibrated for free chlorine inactivation of *C. parvum* oocysts. The $C_{avg}t$ model (Equation 3-12) was used, since the free chlorine residual was almost constant during the test. Model parameters and their 90% confidence limits are listed in Table 7-2. Due to the low inactivation level under the experimental conditions and the intrinsic high variance of infectivity assay, the variance of the parameter estimation was relatively high. Fitness plot of $C_{avg}t$ Chick-Watson model is illustrated in Figure 7-1, suggesting Chick-Watson type model gave a very good fit. Gyürék et al. (1997) analyzed the 22°C data for free chlorine inactivation of *C. parvum* oocysts using a more sophisticated I.g.H. model, which was necessary for some data with very high free chlorine dose (*e.g.* 79.8 mg/L for 120 min). However, using the practical free chlorine doses and contact times, the simple Chick-Watson type model was comparable to the I.g.H. model.

Table 7-2. Chick-Watson model parameters of free chlorine or monochloramine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at 1 to 22°C

Disinfectant	Free chlorine *	Monochloramine *
Temperature (°C)	1 to 22	1 to 22
pH	6	8
Number of trials	19	25
Temperature collection, $\hat{\theta}$ (±90% limits)	1.08 (1.05, 1.14)	1.12 (1.06, 1.47)
$\hat{k}_{22}$ (L/(mg·min)) (±90% limits)	0.00031 (0.00022, 0.00040)	0.00024 (0.00019, 0.00029)
Standard error, s	0.27	0.35
Model constraints: Initial residual C_o (mg/L) Contact time t (min)	$0.9 \leq C_0 \leq 14.2$ $60 \leq t \leq 1032$	$0.9 \leq C_0 \leq 15.2$ $5 \leq t \leq 980$

* Kinetic model see Equation 3-12

The reaction rate constant for the Chick-Watson model was estimated as $\hat{k}_{22}=3.1 \times 10^{-4}$ L/(mg·min) at room temperature. For inactivation of 1.0 log-unit, a $C_{avg}t$ product of 3,000 mg·min/L was required at 22°C. Korich et al. (1990b) studied free chlorine inactivation of *C. parvum* oocysts at pH 7 and temperature 25°C, reported that 80 mg/L of chlorine required approximately 90 min ($C_{avg}t$ product about 7,200 mg·min/L)

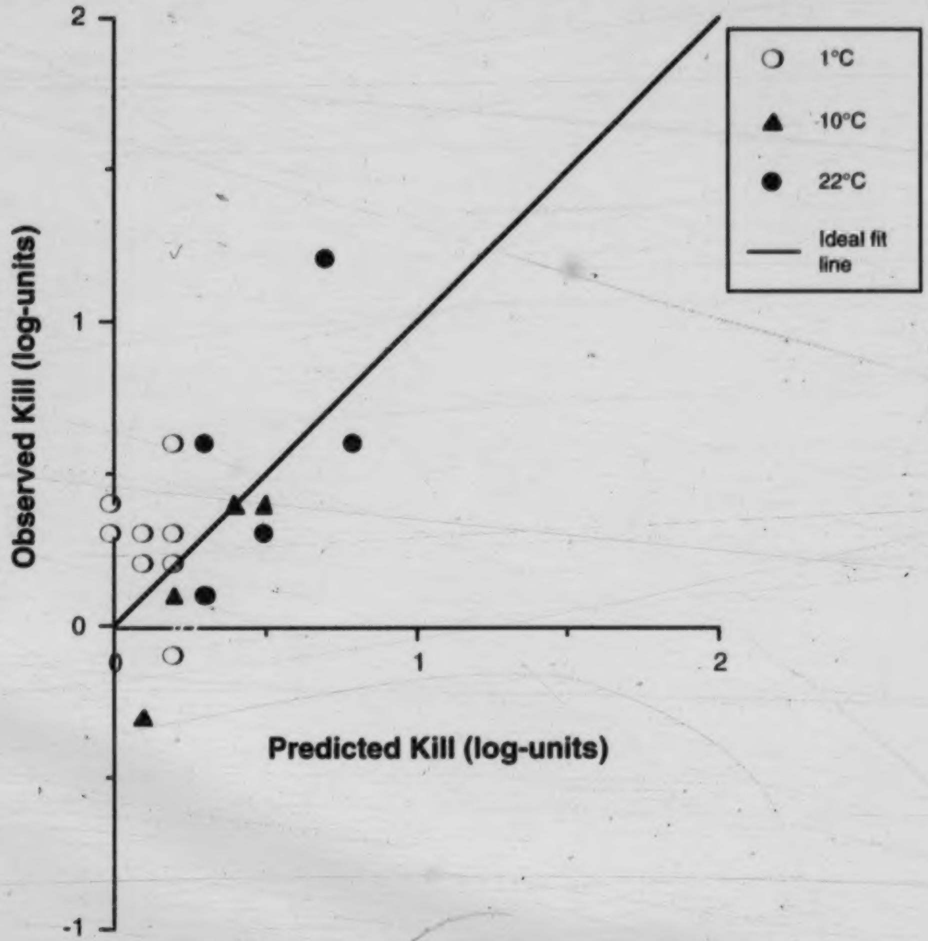


Figure 7-1. Temperature corrected Chick-Watson model fit for free chlorine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1 to 22°C

for at least 99% inactivation. The result from Korich et al. (1990) reasonably agreed with the result from this study. Quinn and Betts (1993) studied the chlorination and the incubation on *C. parvum* oocysts in the tap water (at pH 7.3 and 24°C) using *in vitro* excystation. They reported that a chlorine Ct product up to 150 mg·min/L followed an incubation at room temperature up to 7 days did not affect oocyst viability. Fayer et al. (1995) exposed the *C. parvum* oocysts to sodium hypochlorite at concentrations up to 5.23% and contact times up to 120 min at 21°C. The animal infectivity tests using BALB/c mice showed no reduction in the oocyst infectiousness, suggesting that the hypochlorite ion had little effect on oocyst infectivity even at very high $C_{avg}t$ conditions.

The temperature corrected Chick-Watson model calibrated at pH 6 should also be applied at other pH, provided that the equilibrium of HOCl and OCl^- is adjusted by water pH, so that the hypochlorous acid concentration is used in the $C_{avg}t$ calculation.

7.3.3 Temperature Effect

The temperature coefficient, $\hat{\theta} = 1.08$, was estimated for the temperature corrected Chick-Watson model for free chlorine inactivation at the range of 1 to 22°C. For every 10°C decrease in water temperature the reaction rate constant decreased by a factor or 2.3, which was close to that for free chlorine inactivation of *Giardia* spp. The chlorine Ct requirement for *G. muris* cyst inactivation given by US EPA increased 2-fold for every 10°C of temperature decrease (Malcolm Pirnie Inc. and HDR Engineering Inc. 1991). The activation energy for free chlorine, $E_a = 55$ kJ/mol, and the pre-exponential factor $A = 1.9\times10^6$ 1/min were estimated for free chlorine for 1 to 22°C. In chlorine inactivation of *G. lamblia* cysts, Jarroll et al. (1981) reported an increase of required chlorine Ct product for *G. lambia* cyst inactivation at low temperature. Clark et al.

(1989) reported that the required free chlorine Ct product for 99% inactivation at pH 6 were 70.1, 32.2, and 14.5 (mg·min/L) for 5, 15 and 25°C, respectively. The Ct values increased by a factor of 2 for every 10°C temperature change. Thus, the effect of temperature on chlorine inactivation of *C. parvum* oocysts and *G. lamblia* cysts are comparable, although *C. parvum* oocysts are much more resistant to chlorine.

7.4 RESULTS WITH MONOCHLORAMINE

7.4.1 Microorganism Reduction After Monochloramine Treatment

Data for monochloramine inactivation of *C. parvum* oocysts at pH 8 are summarized in Table 7-3. At 1°C, the monochloramine doses ranged from 3.0 to 4.5 mg/L and the contact times ranged from 240 to 960 min. No kill was observed at 1°C. At 10°C, a kill of 0.2 to 0.7 log-units was observed for a monochloramine dose of 4.5 mg/L for 960 min. At room temperature (22°C), a monochloramine dose of 4.6 mg/L for 960 min resulted in an inactivation of 1.0 log-unit. Some data reported by Gyürék et al. (1997) at room temperature were also re-evaluated. The infectivity results were not adjusted by the individual controls. Data that were either censored or had an extremely high monochloramine dose were not included in the analysis.

Table 7-3. Summary of monochloramine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1 to 22°C

Trial No.	Temp. T (°C)	Initial Cl_2 residual C_o (mg/L)	Final Cl_2 residual C_f (mg/L)	Contact time t (min)	Reactor volume (mL)	Number of oocysts in reactor	Observed Infectivity reduction (log-units)	Predicted infectivity reduction (log-units)
687.2	1	3.0	3.0	240	200	1×10^7	-0.5	0.0
628.1	1	3.0	3.0	240	500	5×10^7	0.3	0.0
816.2	1	3.0	2.6	480	200	4×10^7	0.0	0.0
745.2	1	3.4	3.2	480	200	1×10^7	0.1	0.1
815.1	1	4.5	4.2	480	200	4×10^7	0.3	0.1
816.3	1	3.0	2.9	960	200	4×10^7	-0.6	0.1
628.2	1	3.0	3.0	960	500	5×10^7	0.0	0.1
745.3	1	3.4	3.0	960	200	1×10^7	-0.2	0.1
815.2	1	4.5	4.2	960	200	4×10^7	0.2	0.1
806.1	10	4.5	4.4	480	200	4×10^7	0.7	0.1
632.5	10	0.9	0.9	960	500	5×10^7	-0.1	0.1
754.2	10	3.0	2.0	960	200	1×10^7	-0.2	0.2
806.2	10	4.5	4.4	960	200	4×10^7	0.7	0.3
797.2	10	4.7	4.4	960	200	4×10^7	0.2	0.3
819.2	10	1.5	1.4	980	200	4×10^7	-0.1	0.1
814.1	22	1.5	1.5	460	200	4×10^7	0.3	0.2
796.2	22	4.6	4.2	960	200	4×10^7	1.0	1.0
814.2	22	1.5	1.3	975	200	4×10^7	0.3	0.3
*174**	22	13.3	12.9	5	25	5×10^6	-0.4	0.0
*72**	22	15.2	15.2	13	25	5×10^6	0.1	0.0
*148**	22	10.2	10.0	46	25	5×10^6	0.2	0.1
*55**	22	4.4	4.4	480	25	5×10^6	0.3	0.5
*57**	22	9.7	9.2	480	25	5×10^6	1.4	1.1
*73**	22	15.2	14.2	480	25	5×10^6	1.6	1.7
*42**	22	9.7	9.1	480	25	5×10^6	1.0	1.1

*Source: (Gyürék et al. 1997), control adjustment was not applied in reduction of infectivity.

7.4.2 Kinetic Modeling

The $C_{avg}t$ model (Equation 3-12) was also used for modeling of monochloramine inactivation at different temperatures. Model parameters and their 90% confidence limits are provided in Table 7-2. A fitness plot of model prediction *versus* the observation is shown in Figure 7-2. The plot revealed that, at 1°C, no evident kill was observed for monochloramine inactivation using different doses and contact times. The standard error of model prediction s = 0.35 indicated a very good model prediction. At pH 7 or higher, monochloramine is very stable, suggesting that the model developed for pH 8 should be applicable for other pH values. At 22°C, monochloramine was about two thirds as effective as free chlorine ($\hat{k}_{22}$ = 0.00024 L/(mg·min) for monochloramine and $\hat{k}_{22}$ = 0.00031 L/(mg·min) for free chlorine). Studies (Ward et al. 1984; Wolfe et al. 1985) on heterotrophic bacteria inactivation using chlorine species reported that preformed chloramine was less effective than free chlorine.

Korich et al. (1990b) studied monochloramine inactivation of *C. parvum* oocysts at pH 9 to 10 at room temperature and reported that a Ct product of 7,200 mg·min/L required for at least 90% inactivation. From the Chick-Watson model given by this study, one log-unit of inactivation required a monochloramine $C_{avg}t$ product of 5,000 mg·min/L, which was too high for the common water treatment systems.

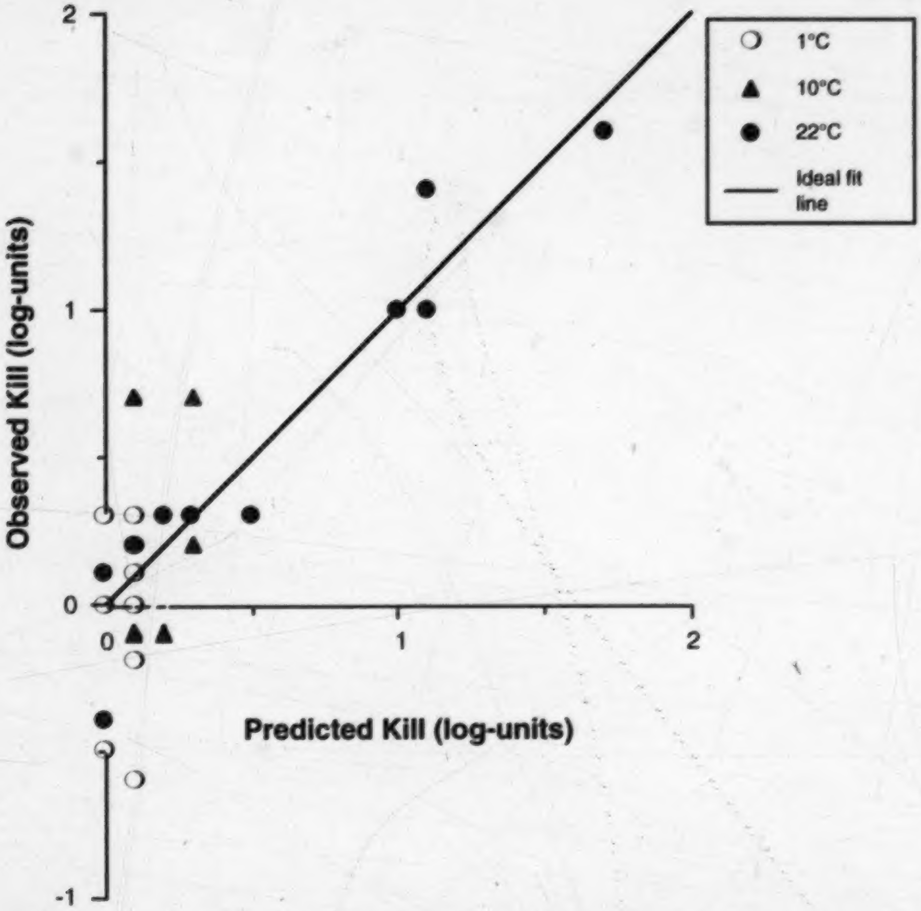


Figure 7-2. Temperature corrected Chick-Watson model fit for monochloramine inactivation of *C. parvum* oocysts in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1 to 22°C

7.4.3 Temperature Effect

The temperature coefficient, $\hat{\theta} = 1.10$, was estimated for monochloramine inactivation of *C. parvum* oocysts at 1 to 22°C. In contrast to free chlorine, the temperature coefficient for monochloramine was slightly higher. For every 10°C decrease in water temperature, the reaction rate constant decreased by a factor of 2.6. The activation energy, $E_a = 65$ kJ/L, and the factor $A = 6.5\times10^7$ 1/min, were estimated for monochloramine inactivation. Meyer et al. (1989) studied the monochloramine inactivation of *G. muris* cysts, and reported that the minimum Ct values required for >99.8 percent inactivation were 610 mg·min/L at 10°C and 385 mg·min/L at 18°C. For every 10°C decrease in temperature, the Ct requirement for *G. muris* cyst inactivation increased by a factor of two.

For ozone, chlorine dioxide, free chlorine and monochloramine, the activation energies were at the same level. Interestingly, the temperature effect was more significant for the "weaker" chemical, which was reported by Wickramanayake et al. (1984a).

7.5 SUMMARY

Free chlorine or monochloramine alone was not very effective for inactivation of *C. parvum* oocyst at low temperatures. At room temperature, less than 1.0 log-unit of inactivation was achieved under practical Ct conditions. The one-parameter average Ct model was suitable for describing free chlorine or monochloramine inactivation of *C. parvum*. At room temperature, the reaction rate constant for free chlorine was about 50% higher than that of monochloramine.

Temperature was found to be a significant factor in free chlorine or monochloramine inactivation of *C. parvum* oocysts. For every 10°C decrease in temperature, the reaction rate constant for decreased by a factor of 2.3 and 2.6 for free chlorine and monochloramine, respectively. At 1°C, free chlorine and monochloramine virtually had no effect on oocyst viability for the practical Ct values.

CHAPTER 8

OZONE FOLLOWED BY FREE CHLORINE

8.1 INTRODUCTION

Ozone has been shown to be one of the most promising chemicals for control of *C. parvum* (Peeters et al. 1989; Korich et al. 1990b; Parker et al. 1993; Finch et al. 1994b; 1997; Gyürék et al. 1999). Chlorine is often used as the secondary chemical treatment after ozone primary disinfection in large water systems. Our previous studies showed that the sequential disinfection using ozone followed by chlorine species enhanced the inactivation of encysted parasites when compared to the same microbial reduction chemicals used singly (Liyanage et al. 1997c; 1998; Finch et al. 2000).

In this chapter, sequential inactivation of *C. parvum* oocysts using ozone and free chlorine was studied systematically. Factors investigated include levels of ozone primary treatment, Ct product of the secondary treatment and water temperature. Empirical models were developed to predict the ozone and free chlorine sequential inactivation.

8.2 EXPERIMENTAL SETTINGS

Experiments were conducted in oxidant demand free 0.05 M phosphate buffer at pH 6, at 1, 10 and 22°C. At each temperature, two levels of ozone pre-treatment, the low (0.4 log-units ozone kill) and the high (1.6 log-units ozone kill), were investigated. The target inactivation level at each temperature was obtained by adjusting the ozone dose and contact time. For the secondary treatment, different doses and contact times were applied. Typically, the chlorine doses were less than 4 mg/L and the contact times were less than 1,000 min.

The effect of ozone primary inactivation and the gross kill by the ozone and free chlorine sequential inactivation were evaluated by serial sampling from a single reactor at different contact times. In the secondary inactivation, samples were taken after short and long contact times to measure the free chlorine effect under different Ct conditions. With a widely separated contact time, serial correlation of the regression error in the kinetic modeling was found not to be very important (Seber and Wild 1989).

8.3 RESULTS OF MICOORGANISM REDUCTION AFTER TREATMENT

Data for ozone followed by free chlorine sequential inactivation of *C. parvum* oocysts are summarized in Table 8-1, Table 8-2, and Table 8-3. In each row of the Tables, two trial numbers were indicated. The first number was for ozone pre-treatment and the second number was for the secondary treatment.

8.3.1 At 1°C

The results for ozone and free chlorine inactivation of *C. parvum* oocysts at 1°C are summarized in Table 8-1. The relationship of the gross survival ratio to the free chlorine $C_{avg}t$ product is illustrated in Figure 8-1.

Table 8-1. Summary of *C. parvum* oocyst inactivation using ozone followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1°C

Trial No.	Primary treatment (ozone)				Secondary treatment (free chlorine)					Total		
	C_o (mg/L)	C_f (mg/L)	t (min)	Observed I_{r1} (log-units)	C_o (mg/L)	C_f (mg/L)	t (min)	$C_{avg}t$ (mg·min/L)	I_{r2}* (log-units)	Observed I_r (log-units)	Synergy† (log-units)	Predicted kill (log-units)
700.1 700.2	0.9	0.6	15	-0.5	2.9	2.5	240	648	0.0	0.2	0.7	-0.4
671.1 671.2	1.0	0.9	15	0.6	3.0	2.8	240	696	0.0	1.3	0.7	0.7
733.2 733.3	1.0	0.7	15	0.4	4.1	3.8	480	1890	0.1	1.4	0.9	0.6
671.1 671.3	1.0	0.9	15	0.6	3.0	2.5	960	2640	0.2	1.2	0.4	0.9
666.1 666.2	1.9	1.5	30	2.5	0.7	0.6	240	156	0.0	2.5	0.0	2.6
666.1 666.3	1.9	1.5	30	2.5	0.7	0.5	960	576	0.0	3.2	0.7	2.8
736.1 736.2	1.7	1.3	30	1.3	1.5	1.0	480	600	0.0	2.0	0.7	1.6
665.1 665.2	1.9	1.5	30	2.4	2.7	2.6	240	636	0.0	2.6	0.2	2.7
699.1 699.2	2.1	1.4	30	1.5	2.9	2.5	240	648	0.0	1.5	0.0	1.8
736.1 736.3	1.7	1.3	30	1.3	1.5	0.9	1020	1220	0.1	2.7	1.3	1.8
661.1 661.3	1.9	1.7	25	0.9	2.8	2.6	480	1300	0.1	2.1	1.1	1.5
734.1 734.2	1.7	1.3	30	1.9	3.9	3.6	480	1800	0.1	2.6	0.6	2.7
665.1 665.3	1.9	1.5	30	2.4	2.7	2.4	960	2450	0.2	3.3	0.7	3.5
699.1 699.3	2.1	1.4	30	1.5	2.9	2.5	920	2480	0.2	2.5	0.8	2.6
734.1 734.3	1.7	1.3	30	1.9	3.9	3.4	1020	3720	0.2	3.4	1.3	3.6

* Free chlorine kill was estimated by the Chick-Watson model (Table 7-3);
† Synergy was calculated using Equation 3-3.

There were two levels of ozone primary treatment. The low level ozone pre-treatment used an ozone dose of 1 mg/L for 15 min, resulting in an inactivation ranging from -0.5 to 0.6 log-units. The average ozone inactivation of these triplicate tests was 0.4 log-units. For the secondary treatment, the free chlorine $C_{avg}t$ products were from 648 to 2,460 mg·min/L, resulting in a gross kill from 0.2 to 1.4 log-units for the sequential treatment. About 0.4 to 0.9 log-units of synergy was observed.

The high level ozone pre-treatment used a dose of 2 mg/L for 30 min, which resulted in an average primary kill of about 1.7 log-units. The free chlorine $C_{avg}t$ for the secondary disinfection ranged from 156 to 3,720 mg·min/L, resulting in a gross kill from 1.5 to 3.4 log-units. The gross kill by ozone and free chlorine disinfection increased linearly with the free chlorine $C_{avg}t$ product (Figure 8-1). The gross kill of 3.0 log-units requried a free chlorine $C_{avg}t$ product about 3,500 mg·min/L for the ozone pre-treatment of 1.7 log-units.

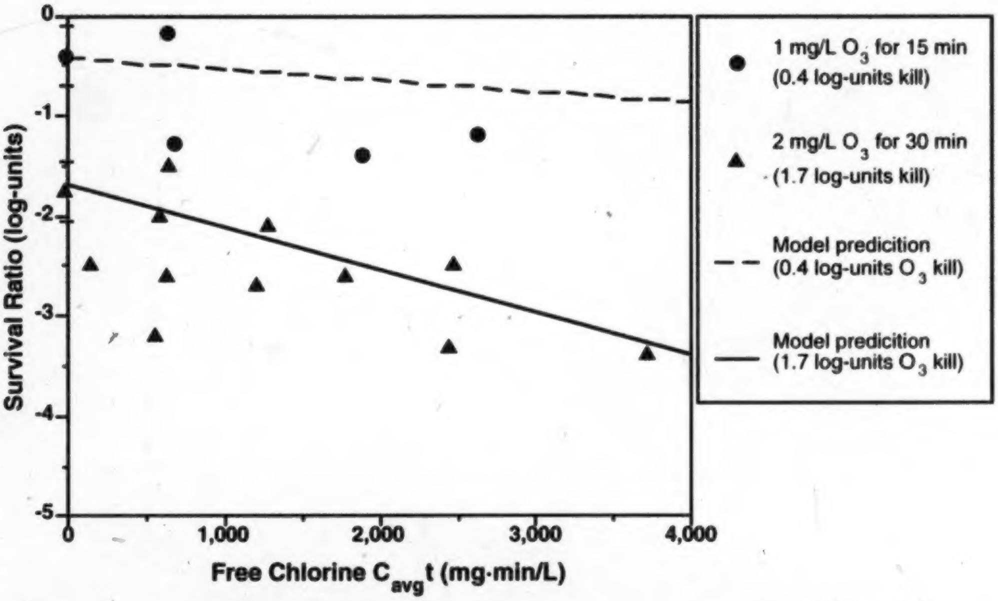


Figure 8-1. Survival ratios of *C. parvum* oocysts as a function of free chlorine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1°C

8.3.2 At 10°C

Results for ozone and free chlorine sequential treatment of *C. parvum* oocysts at 10°C are summarized in Table 8-2. Survival curves under different free chlorine $C_{avg}t$ products are illustrated in Figure 8-2. Two levels of ozone pre-treatment were also investigated. At the low level of ozone pre-treatment, an ozone dose of 1.0 mg/L for about 5 min resulted in an inactivation of 0.4 to 0.6 log-units. In the free chlorine secondary treatment, a gross kill of 1.4 log-units was observed at a high free chlorine $C_{avg}t$ product of 2,660 mg·min/L. Some synergistic effect was observed at high free chlorine $C_{avg}t$.

Table 8-2. Summary of *C. parvum* oocyst inactivation using ozone followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 10°C

Trial No.	Primary treatment (ozone)				Secondary treatment (free chlorine)					Total		
	C_o (mg/L)	C_f (mg/L)	t (min)	Observed I_{r1} (log-units)	C_o (mg/L)	C_f (mg/L)	t (min)	$C_{avg}t$ (mg·min/L)	I_{r2}* (log-units)	Observed I_r (log-units)	Synergy† (log-units)	Predicted kill (log-units)
750.2 750.3	1.0	0.9	5	0.4	2.6	2.2	480	1150	0.1	0.7	0.2	0.8
750.2 750.4	1.0	0.9	5	0.4	2.6	2.0	960	2210	0.3	0.8	0.1	1.2
710.2 710.3	1.0	0.8	7	0.6	3.0	2.9	900	2660	0.3	1.4	0.5	1.6
731.1 731.2	1.1	0.8	15	1.6	1.5	1.0	420	525	0.1	1.1	-0.6	2.0
711.1 711.2	1.5	1.2	15	1.3	3.0	2.9	240	708	0.1	2.3	0.9	1.8
731.1 731.3	1.1	0.8	15	1.6	1.5	0.9	960	1150	0.1	2.4	0.7	2.4
731.1 732.2	1.2	0.9	15	1.6	3.6	3.0	420	1390	0.2	2.3	0.5	2.6
711.1 711.3	1.5	1.2	15	1.3	3.0	2.9	960	2830	0.3	3.6	2.0	3.4
732.1 732.3	1.2	0.9	15	1.6	3.6	2.7	960	3020	0.4	3.6	1.6	3.8

* Free chlorine kill was estimated by the Chick-Watson model (Table 7-3);

† Synergy was calculated using Equation 3-3

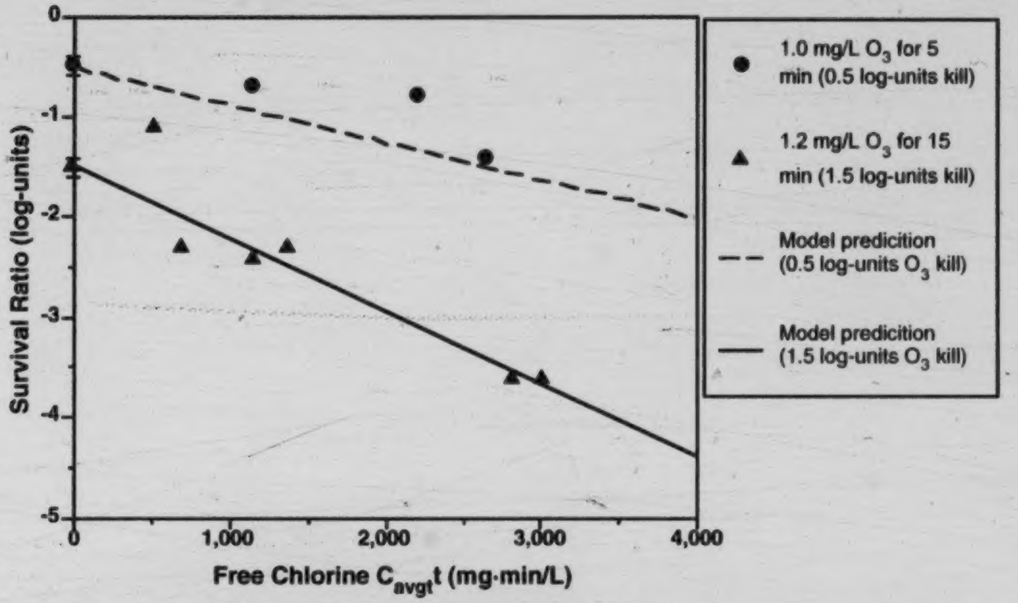


Figure 8-2. Survival ratios of *C. parvum* oocysts as a function of free chlorine C_{avg}t after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 10°C

The high level of ozone pre-treatment using an ozone dose of 1.2 mg/L ozone for 15 min, resulted in a primary kill from 1.3 to 1.6 log-units (1.5 log-units in average). For the secondary treatment, 3 mg/L of free chlorine for 960 min ($C_{avg}t$ = 3,830 mg·min/L) resulted in a gross kill of 3.6 log-units. Considering that free chlorine alone only has trivial effect on oocyst viability, the synergistic effect at high free chlorine C_{avg}t was very significant.

8.3.3 At 22°C

The room temperature (22°C) data are summarized in Table 8-3. The dose and contact time for ozone pre-treatment at 22°C were much lower than those used at lower temperatures. Three trials were conducted for low ozone pre-treatment using the dose of 0.4 mg/L for a contact time of 4 min, resulting in an inactivation of 0.1 to 0.4 log-units. For the free chlorine secondary treatment, evident synergistic effect was observed. A 2.2 log-units synergistic effect was observed with a free chlorine dose of 2.7 mg/L for 400 min. Addition of free chlorine $C_{avg}t$ product of 3,210 mg·min/L resulted in a gross kill greater than 4.4 log-units, which was beyond the detection limit of the animal infectivity assay.

At the high ozone pre-treatment, an ozone dose of 1.0 mg/L for 5 min induced inactivation from 1.3 to 1.8 log-units (1.5 log-units in average). The $C_{avg}t$ in the free chlorine secondary treatment ranged from 135 to 2,260 mg·min/L. The inactiation by free chlorine alone was less to 0.7 log-units estimated by the Chick-Watson model. At room temperature, the ozone pre-treatment enhanced the free chlorine performance dramatically. At low free chlorine $C_{avg}t$ (less than 345 mg·min/L), the synergistic effect was not evident. However, pronouced synergy was observed for higher free chlorine doses. A 1.4 log-units of synergy was observed at a free chlorine $C_{avg}t$ of 675 mg·min/L. Gross kill by the ozone and free chlorine sequential inactivation was greater than 4.1 log-units, which approached the detection limit of the animal infectivity assay.

Table 8-3. Summary of *C. parvum* oocyst inactivation using ozone followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 22°C

Trial No.	Primary treatment (ozone)				Secondary treatment (free chlorine)					Total		
	C_o (mg/L)	C_f (mg/L)	t (min)	Observed I_{r1} (log-units)	C_o (mg/L)	C_f (mg/L)	t (min)	$C_{avg}t$ (mg·min/L)	I_{r2}* (log-units)	Observed I_r (log-units)	Synergy† (log-units)	Predicted kill (log-units)
741.2 741.3	0.3	0.3	4	0.1	1.10	0.30	400	280	0.1	0.9	0.7	0.6
741.2 741.4	0.3	0.3	4	0.1	1.10	0,10	930	558	0.2	0.8	0.5	1.1
742.1 742.2	0.3	0.3	4	0.3	2.70	2.20	260	637	0.2	2.3	1.8	1.5
742.1 742.3	0.3	0.3	4	0.3	2.70	2.10	400	960	0.3	2.8	2.2	2.1
737.2 737.3	0.4	0.3	4	0.4	3.6	2.7	510	1610	0.5	2.7	1.8	3.3
737.2 737.4	0.4	0.3	4	0.4	3.6	2.7	1020	3210	1.0	> 4.4	> 3.0	6.3
716.1 716.2	0.9	0.8	5	1.8	0.4	0.0	660	135	0.0	1.5	-0.3	2.0
729.2 729.3	0.9	0.8	4	1.5	1.3	1.0	300	345	0.1	1.8	0.2	2.0
730.1 730.2	0.9	0.8	4	1.5	2.5	2.0	300	675	0.2	3.1	1.4	2.5
729.2 729.4	0.9	0.8	4	1.5	1.3	0.7	870	870	0.3	2.8	1.0	2.8
752.1 752.3	1.0	0.9	5	1.6	2.40	1.90	480	1030	0.3	2.7	0.8	3.1
717.2 717.3	0.9	0.7	5	1.3	2.0	1.4	660	1120	0.3	3.4	1.8	2.9
719.2 719.3	1.0	0.7	5	1.5	2.7	2.3	480	1200	0.4	> 3.1	> 1.2	3.2
730.1 730.3	0.9	0.8	4	1.5	2.5	1.6	870	1780	0.6	3.9	1.8	4.1
719.2 719.4	1.0	0.7	5	1.5	2.7	2.0	960	2260	0.7	> 4.1	> 1.9	4.8

* Free chlorine kill was estimated by the Chick-Watson model (Table 7-3);

† Synergy was calculated using Equation 3-3.

The survival curves for the secondary treatment were shown in Figure 8-3. The gross kill of ozone and free chlorine inactivation increased linearly with the free chlorine $C_{avg}t$ product. At room temperature, the level of ozone pre-treatment was not very important to the gross kill. Maintaining a certain level of free chlorine for a long contact time is necessary for synergy. In some trials (*e.g.* trial Nos. 741.2 and 741.4) with low intial free chlorine concentration, residual chlorine became very low even before the end of contact time. Thus, lower kills were observed for those trials.

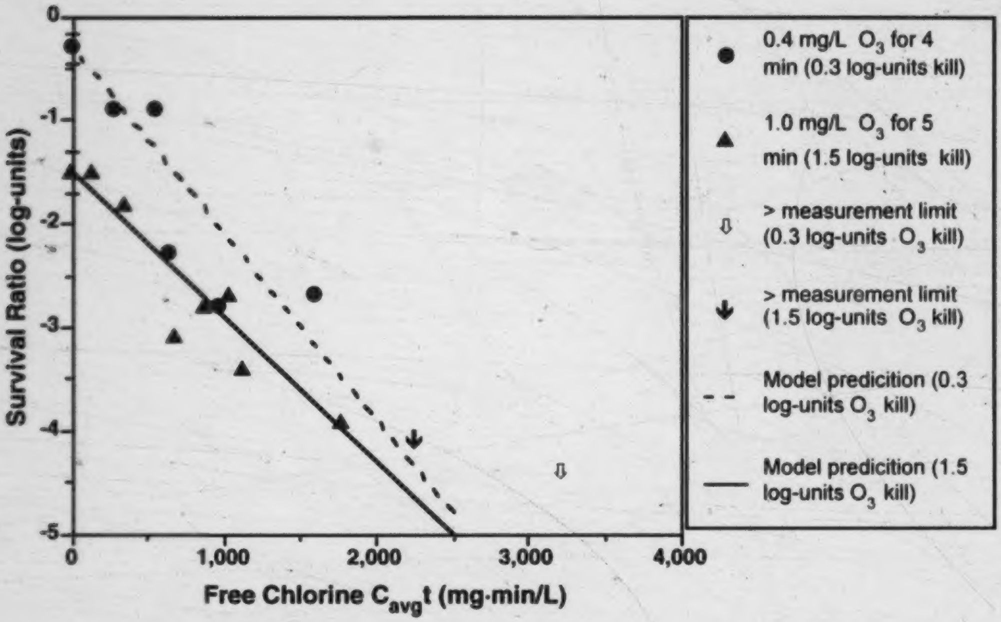


Figure 8-3. Survival ratios of *C. parvum* oocysts as a function of free chlorine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 22°C

8.4 CHICK-WATSON MODELS

From the survival curves for ozone and free chlorne sequential inativation at different temperatures (Figure 8-4 and Figure 8-5), gross kill increased linearly with the free chlorine $C_{avg}t$ product. A certain free chlorine $C_{avg}t$ value was required for some synergistic effect to be observed. For the same level of ozone pre-treatment, the slope of the survival curves at different temperatures was steeper at higher temperatures, indicating higher kill-rates at higher temperature. The linear Chick-Watson model

(Equation 3-15) was used to estimate the gross kill of ozone and free chlorine sequential inactivation. An individual model calibration was applied for each ozone pre-treatment level, and the effect of temperature on the reaction constant was adjusted using the van't Hoff-Arrhenius relationship. Model parameters and their 90% confidence intervals are shown in Table 8-4. Fitness plots of the observed value and model prediction are also indicated in Figure 8-4 and Figure 8-5.

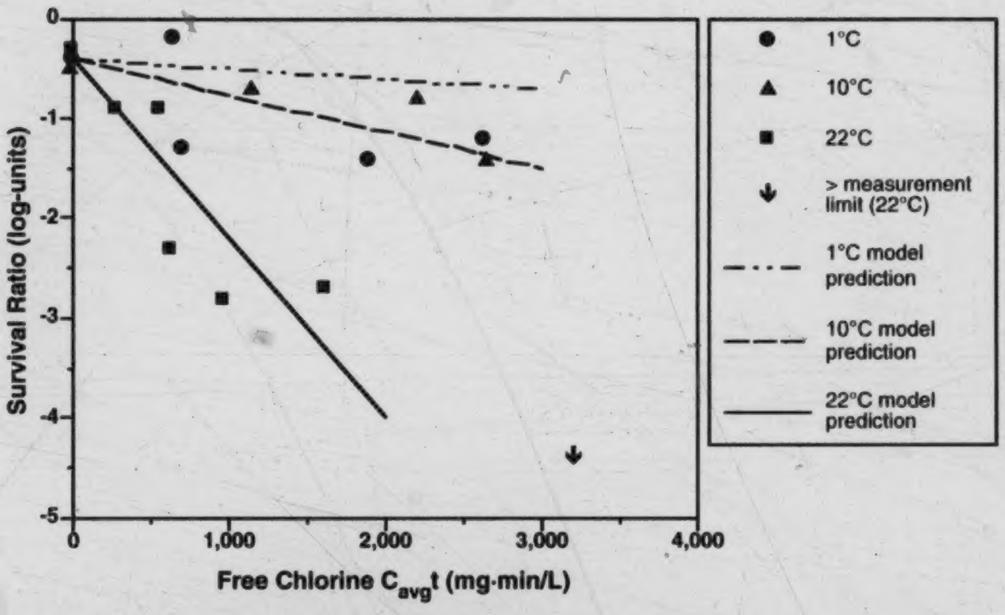


Figure 8-4. Chick-Watson model prediction on inactivation of *C. parvum* oocysts using 0.4 log-units of ozone pre-treatment kill followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1, 10 and 22°C

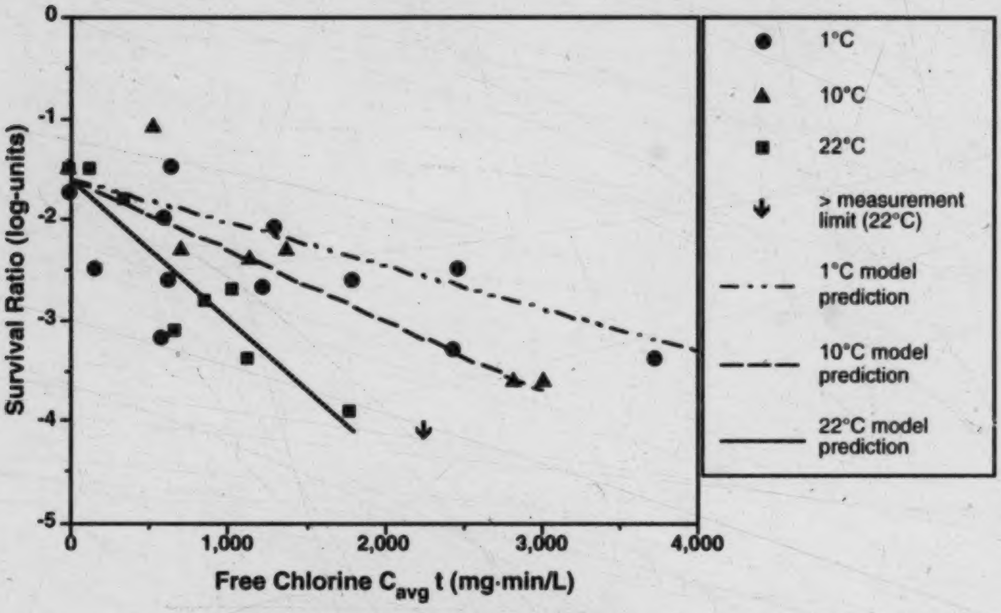


Figure 8-5. Chick-Watson model prediction on inactivation of *C. parvum* oocysts using 1.6 log-units of ozone pre-treatment kill followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1, 10 and 22°C

The linear Chick-Watson model of ozone and free chlorine sequential inactivation indicated that a certain free chlorine $C_{avg}t$ was essential for obtaining some observable synergy. For the primary ozone treatment induced 1.6 log-units of inactivation at pH 6, 1 log-unit of synergistic effect required a secondary free chlorine $C_{avg}t$ product about 800 mg·min/L, 1,500 mg·min/L and 2,500 mg·min/L for 22, 10 and 1°C, respectively.

Table 8-4. Chick-Watson model parameters for sequential inactivation of *C. parvum* oocysts using ozone followed by free chlorine in oxidant demand-free 0.05 M phosphate buffer at pH 6 and 1 to 22°C

Secondary chemical	Free Chlorine *	
Ozone pre-treatment levels (log-units)	0.4	1.6
Number of data points	13	28
Temperature coefficient $\hat{\theta}$ (90% limits)	1.14 (1.10, 1.26)	1.06 (1.05, 1.07)
$\hat{k}_{22}$ (L/(mg·min)) (90% limits)	0.0018 (0.0014, 0.0022)	0.0014 (0.0013, 0.0016)
Error σ	0.54	0.38
Model constraints for secondary disinfection Initial residual C_o (mg/L) Contact time t (min)	$0.1 \leq C_o \leq 4.1$ $240 \leq t \leq 1020$	$0.1 \leq C_o \leq 3.9$ $240 \leq t \leq 1020$

* Kinetic model see Equation 3-15

Ransome et al. (1993) studied the inactivation of *C. parvum* oocysts using ozone followed by free chlorine in buffered spring water at pH 7, and temperature 10°C. Using an initial ozone residual of 1.2 mg/L for 10 min followed by an initial chlorine concentration of 0.5 mg/L applied for 30 min (free chlorine $C_{avg}t$ about 15 mg·min/L), they reported that the secondary free chlorine did not contribute to additional inactivation of *C. parvum* oocysts. Models in this study support their observation in that no evident synergistic effect should be observed at such a low free chlorine $C_{avg}t$ product.

8.5 EFFECT OF OZONE PRE-TREATMENT

The ozone pre-treatment greatly increased the free chlorine microorganism reduction rate constant for *C. parvum* oocyst inactivation. For 0.4 log-units of ozone pre-treatment at 22°C, the microorganism reduction rate constant of the free chlorine secondary treatment, $\hat{k}_{22}$=0.0018 L/(mg·min), was about 6 times that of free chlorine alone, $\hat{k}_{22}$=0.00031 L/(mg·min). For 1.6 log-units of ozone pre-treatment at 22°C, the reaction rate constant of the free chlorine secondary treatment, $\hat{k}_{22}$=0.0014 L/(mg·min), was about 4.5 times that for free chlorine alone. The significant enhancement of the free chlorine inactivation rate after ozone pre-treatment indicated the evidence of synergy in ozone/free chlorine sequential treatment.

Comparing the two levels of ozone pre-treatment at 22°C, the microorganism reduction rate constants for the free chlorine secondary treatment were slightly higher for lower level of ozone pre-treatment. Under the same secondary treatment, a higher level of ozone pre-treatment resulted in a higher overall kill. However, in terms of synergistic effect, the relationship between ozone pre-treatment and synergy was more complicated. At 22°C, a low ozone pre-treatment of 0.4 log-units induced a higher synergistic effect than the pre-treatment of 1.6 log-units, while at 1 and 10°C the synergistic effect was not influenced by the pre-treatment. The results showed that in some cases a lower level of ozone pre-treatment might result in a higher synergistic effect. Nevertheless, for the same free chlorine secondary treatment, higher gross kill was always observed at higher levels of ozone pre-treatment.

8.6 EFFECT OF TEMPERATURE

Temperature was a critical variable for both single and sequential inactivation of *C. parvum* oocysts. The required $C_{avg}t$ for the same target inactivation level by ozone pre-treatment was much higher at low temperatures. In the sequential inactivation, high temperature was a favorable factor for both gross kill and synergy. The effect of temperature was more pronounced for lower level of ozone pre-treatment. Less synergy was observed for low level of ozone pre-treatment at 1 and 10°C in contrast to the room temperature where significant synergy was observed.

For 0.4 log-units of ozone pre-treatment, the microorganism reduction rate constant decreased by a factor of 3.7 for every 10°C temperature decrease. For 1.6 log-units of ozone pre-treatment, the microorganism reduction rate constant decreased by a factor of 1.8 for every 10°C temperature decrease. The activation energies were 88 and 38 kJ/mol for low and high level of ozone pre-treatment, respectively.

8.7 HYPOTHESIS OF SYNERGY

A hypothesis for the mechanism of synergy would be that the strong oxidants in the primary treatment increase the permeability of the oocyst wall by physically damaging or altering its surface properties, so the secondary disinfectants can easily penetrate into the interior of the oocysts to inactivate the organisms. It is known that the physical damage of oocyst wall by sand shaking increased the reaction rate of the following free chlorine inactivation (Parker et al. 1995).

The dormant *C. parvum* sporozoites in the oocysts are protected by a thick outer wall. The oocysts thick wall has two distinct layers (Fayer and Ungar 1986) and has highly complex lattice structure (Harris and Petry 1999), which make them extremely resistant to chemical penetration and mechanical disruption. Both free chlorine and monochloramine are very effective microbicides. However, without ozone pre-treatment, diffusion of these chemicals through the oocyst wall is the rate limiting step in the diffusion-reaction process. Since ozone is a very strong oxidant, and it can oxidize many organic materials including lipid and proteins, it is postulated that the ozone pre-treatment increased oocyst wall permeability by weakening the oocyst wall.

The achievable synergy in the sequential treatment actually depends on the size of the sub-lethally injured population of the oocysts and the extent of sub-lethal injury. Usually a stronger oxidant is better for pre-treatment, since it is less selective in the microbial inactivation and tends to induce bigger sub-lethally injured population in a short period of time. For example, more synergy was observed at high temperature since the oxidizing ability of the primary disinfectant was stronger. Other factors such as oxidant concentration also affect the oxidant effectiveness, so they also affect the synergy.

The level of primary treatment also has direct effect on synergy. Ideally, the ozone primary oxidant should be strong enough to cause to sub-lethal injury. However, if the pre-treatment level is too high, it will inactivate most of the oocysts, leaving less room for the secondary treatment. This may explain the findings in ozone followed by free chlorine sequential inactivation at 22°C, where more synergy was observed at lower level of ozone pre-treatment.

Some studies reported that synergy existed when some metal ions were combined with oxidants in microorganism reduction. For example, Lin et al. (1996; 1998) reported that a combination of copper and silver enhanced the rate of inactivation to *Legionella pneumophila* and *Mycobacterium avium*. Copper is a cell wall active agent that can increase the permeability whereas silver ions prohibit the protein and enzyme synthesis (Domek et al. 1984). Yahya et al. (1992) reported that a combination of copper, silver and free chlorine acted synergistically in the inactivation of coliphage MS-2 and poliovirus type 1. However, Abad et al. (1994) studied the inactivation of human enteric virus such as hepetitis A virus, human rotavirus, human adenovirus and poliovirus, and reported no synergy using the combination of copper, silver ion and free chlorine. The discrepancy among different microorganisms may due to the difference in their cell wall proteins. Straub et al. (1995) studied the combination of monochloramine and copper (in the form of cupric chloride) in the inactivation of *E. coli* and MS-2 coliphage, and reported synergy for both organisms. A 6 log-unit inactivation of *E.coli* was observed after an exposure to 2.5 mg/L monochloramine plus 0.8 or 0.4 mg/L cupric chloride for 20 min, whereas for the same level of inactivation using monochloramine alone required a dose of 5 mg/L for 60 min. The combination of a sub-lethal dose of silver with hydrogen peroxide also enhanced the inactivation of *E. coli* (Pedahzur et al. 1995; 1997).

8.8 SUMMARY

Sequential inactivation using ozone and free chlorine was found to be very effective in *C. parvum* oocyst inactivation and significant synergy was observed under selected conditions. The gross kill for ozone and free chlorine sequential disinfection increased approximately linearly with the free chlorine $C_{avg}t$ product.

Water temperature was a critical factor for ozone and free chlorine sequential disinfection. Higher water temperature resulted in a higher gross kill and higher synergy. The effect of temperature on the model was adjusted using van't Hoff-Arrhenius relationship. For a 1.6 log-units of inactivation, 10°C increase in the water temperature resulted in the increase of the free chlorine reaction rate constant by a factor of 1.8. Given the ozone pre-treatment provide 1.6 log-unit of inactivation at pH 6, the free chlorine $C_{avg}t$ products required for 3.0 log-units gross kill were about 1,000, 2,000 and 3,500 mg·min/L for 22, 10 and 1°C, respectively.

The level of ozone pre-treatment also affected the synergy. The free chlorine reaction rate constant after ozone pre-treatment increased about 6 to 4.5 times for 0.4 and 1.6 log-units of ozone primary kills, respectively. Higher ozone pre-treatment resulted in a higher gross kill in the sequential treatment. At 22°C, the synergistic effect was higher for 0.4 log-units of primary inactivation than that for 1.6 log-units of inactivation.

CHAPTER 9

OZONE FOLLOWED BY MONOCHLORAMINE

9.1 INTRODUCTION

Chloramines are often used as the secondary chemicals after ozone primary treatment. In contrast to free chlorine, chloramines have the benefit of effectiveness in controlling biofilms, an ability to reach remote areas of water distribution systems and a tendency to form less THMs and other disinfection by-products (Kirmeyer et al. 1993).

The previous chapter on ozone with free chlorine sequential inactivation of *C. parvum* oocysts showed that water temperature was a crucial factor for sequential treatment, and the gross kill was shown to increase linearly with the free chlorine Ct products. The objectives of this chapter were to examine the inactivation of oocysts treated with ozone followed by monochloramine at different temperatures and levels of ozone pre-treatment, and to identify the important factors for synergy in ozone and monochloramine sequential treatment.

9.2 EXPERIMENTAL SETTINGS

The experimental design for ozone followed by monochloramine was similar to that for ozone and free chlorine sequential inactivation. Experiments were conducted in oxidant demand-free 0.05 M phosphate buffer at pH 8 and temperature of 1, 10 and 22°C.

The ozone doses and contact times were designed the same as the ozone and free chlorine study in the last chapter, however, due to the higher ozone decay at higher pH values, the ozone kills at pH 8 were slightly lower. At each temperature, the ozone pre-treatments were targeted at 0.4 and 1.4 log-units of inactivation low and high level of primary kill, respectively.

9.3 RESULTS OF MICROORGANISM REDUCTION AFTER TREATMENT

9.3.1 At 1°C

Results for ozone followed by monochloramine treatment of *C. parvum* oocysts at 1°C and pH 8 are summarized in Table 9-1. Two levels of ozone pre-treatment were investigated. At the low level of ozone pre-treatment, an ozone primary kill of -0.2 to 0.8 log-units was induced by the ozone dose of 1.1 mg/L and 15 min contact time. In the monochloramine secondary treatment, the observed gross kills were below 1.4 log-units for monochloramine $C_{avg}t$ products of up to 2,910 mg·min/L. Some extra benefit was observed at high monochloramine $C_{avg}t$.

At the high level of ozone pre-treatment, an ozone dose of 2 mg/L ozone for 30 min caused an inactivation of 1.4 log-units on average. For the following monochloramine treatment, the $C_{avg}t$ product of the monochloramine ranged from 138 to 3,360 mg·min/L. Gross kills of the ozone and monochloramine treatment ranged from

Table 9-1. Summary of *C. parvum* oocyst inactivation using ozone followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1°C

Trial No.	Primary treatment (ozone)				Secondary treatment (Monochloramine)					Total		
	C_o (mg/L)	C_f (mg/L)	t (min)	Observed I_{r1} (log-units)	C_o (mg/L)	C_f (mg/L)	t (min)	$C_{avg}t$ (mg·min/L)	I_{r2} † (log-units)	Observed I_r (log-units)	Synergy‡ (log-units)	Predicted kill (log-units)
677.1 677.2	1.2	0.8	15	0.8	3.0	2.9	240	708	0.0	0.7	-0.1	1.0
683.1 683.2	1.1	0.8	15	0.4 *	3.1	3.1	240	738	0.0	0.5	0.4	0.6
677.1 677.3	1.2	0.8	15	0.8	3.0	2.9	960	2830	0.1	1.4	0.5	1.6
688.2 688.3	1.0	0.4	15	-0.2	3.3	3.1	910	2910	0.1	0.6	0.7	0.6
724.2 724.3	1.9	0.8	30	1.4	1.2	1.1	120	138	0.0	1.1	-0.3	1.5
725.1 725.2	1.9	0.8	30	1.4	2.1	2.0	120	246	0.0	1.6	0.2	1.5
701.1 701.2	1.9	1.0	30	0.8	1.0	1.0	360	351	0.0	1.7	0.9	1.0
721.2 721.3	2.5	0.9	30	1.5	1.2	1.1	480	552	0.0	2.1	0.6	1.8
674.1 674.2	1.9	0.5	30	0.9	3.0	2.9	240	708	0.0	1.7	0.8	1.3
686.1 686.2	1.9	1.0	30	1.4	3.3	3.2	240	780	0.0	1.8	0.4	1.8
701.1 701.3	1.9	1.0	30	0.8	1.0	1.0	960	936	0.0	1.8	1.0	1.3
721.2 721.4	2.5	0.9	30	1.5	1.2	1.1	960	1100	0.0	3.1	1.6	2.1
724.2 724.4	1.9	0.8	30	1.4	1.2	1.1	960	1100	0.0	2.1	0.7	2.0
725.1 725.3	1.9	0.8	30	1.4	2.1	2.0	960	1970	0.0	2.3	0.9	2.5
674.1 674.3	1.9	0.5	30	0.9	3.0	2.9	960	2830	0.1	2.5	1.5	2.4
686.1 686.3	1.9	1.0	30	1.4	3.3	3.2	900	2930	0.1	3.1	1.6	3.0
682.1 682.3	2.1	1.3	30	1.6 *	3.1	3.1	960	2970	0.1	2.5	1.3	3.2
720.2 720.5	2.5	2.0	30	2.3	3.6	3.4	960	3360	0.1	4.1	1.7	4.1

* Ozone kill estimated by I.g.H. model based on the concentration and contact time (Table 5-4);

† Monochloramine kill was estimated by the Chick-Watson model (Table 7-3);

‡ Synergy was calculated using Equation 3-3.

1.1 to 4.1 log-units. The gross kill reached 3.0 log-units at the monochloramine $C_{avg}t$ of about 3,000 mg·min/L. Without the ozone pre-treatment, monochloramine alone did not have a significant inactivation effect on *C. parvum* oocysts at 1°C. The ozone pre-treatment greatly enhanced the inactivation of *C. parvum* oocysts using monochloramine. A significant synergy was observed at high secondary $C_{avg}t$ (3,000 mg·min/L). The survival curves after the secondary treatment (Figure 9-1) showed that the oocyst survival ratio decreased linearly with the monochloramine $C_{avg}t$ product.

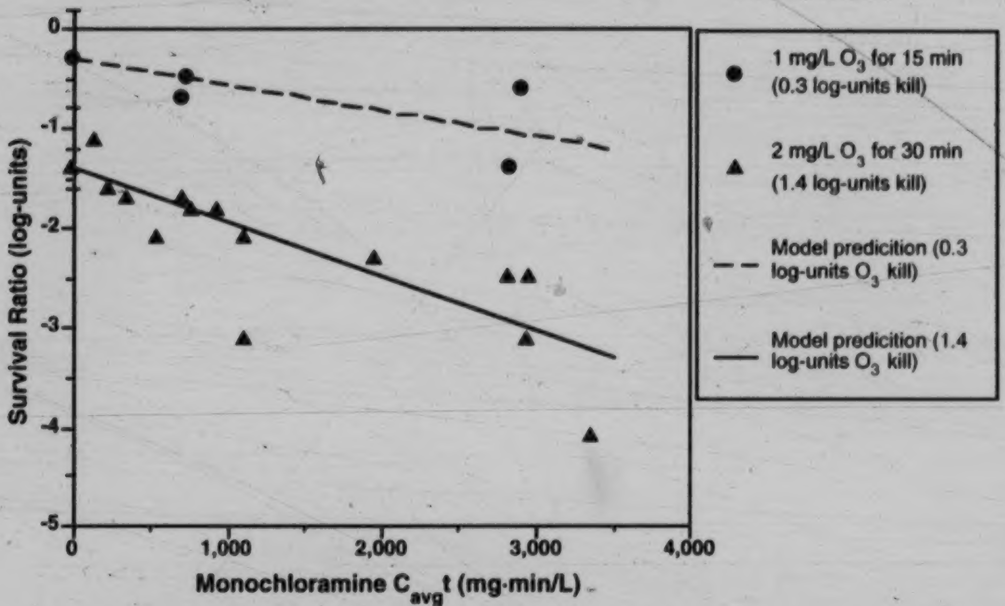


Figure 9-1. Survival ratios of *C. parvum* oocysts as a function of monochloramine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1°C

9.3.2 At 10°C

Data for ozone with monochloramine inactivation at 10°C and pH 8 are summarized in Table 9-2. At the low level of ozone pre-treatment, the observed ozone primary kill was 0.1 log-units for an ozone dose of 0.9 mg/L and contact time 5 min. In the monochloramine secondary treatment, monochloramine $C_{avg}t$ ranged from 660 to 2,660 mg·min/L, resulting in a gross kill of 0.2 to 1.4 log-units. Evident synergy was observed at high monochloramine $C_{avg}t$. At high level of ozone pre-treatment, an ozone dose of 1.2 mg/L for 15 min induced an average ozone kill of 1.4 log-units. $C_{avg}t$ products of the secondary monochloramine treatment were from 696 to 2,970 mg·min/L. Gross kills of ozone with monochloramine treatment at 10°C ranged from 2.1 to 3.4 log-units, and 0.5 to 1.7 log-units of synergy were observed for the high ozone pre-treatment.

The survival ratios of *C. parvum* oocysts under ozone and monochloramine treatment are presented in Figure 9-2. For both low and high level of ozone pre-treatment, the gross kills increased linearly with the $C_{avg}t$ product of the secondary

Table 9-2. Summary of *C. parvum* oocyst inactivation using ozone followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 10°C

Trial No.	Primary treatment (ozone)				Secondary treatment (Monochloramine)					Total		
	C_o (mg/L)	C_f (mg/L)	t (min)	Observed I_{t1} (log-units)	C_o (mg/L)	C_f (mg/L)	t (min)	$C_{avg}t$ (mg·min/L)	I_{t2} † (log-units)	Observed I_t (log-units)	Synergy‡ (log-units)	Predicted kill (log-units)
743.2 743.3	0.9	0.7	5	0.1	1.4	1.4	480	660	0.0	0.7	0.6	0.4
705.1 705.2	0.9	0.6	5	0.1	3.0	2.9	240	708	0.0	0.2	0.1	0.4
743.2 743.4	0.9	0.7	5	0.1	1.4	1.4	955	1310	0.1	1.4	1.2	0.7
705.1 705.3	0.9	0.6	5	0.1	3.0	2.9	900	2660	0.2	1.2	0.9	1.3
744.1 744.2	1.1	0.6	15	1.4	1.5	1.4	480	696	0.0	2.6	1.2	2.0
704.1 704.2	1.2	0.6	15	1.2	3.0	2.9	240	708	0.0	2.4	1.2	1.8
684.1 684.2	1.2	0.9	15	1.6 *	3.1	3.1	240	742	0.0	2.1	1.0	2.3
744.1 744.3	1.1	0.6	15	1.4	1.5	1.4	965	1400	0.1	3.2	1.7	2.6
728.2 728.3	1.2	0.5	15	1.6	3.1	3.1	480	1480	0.1	2.4	0.7	2.9
704.1 704.3	1.2	0.6	15	1.2	3.0	2.9	900	2660	0.2	3.1	1.7	3.5
728.2 728.4	1.2	0.5	15	1.6	3.1	3.1	960	2970	0.2	3.4	1.6	4.2

* Ozone kill estimated by I.g.H. model based on the concentration and contact time (Table 5-4);

† Monochloramine kill was estimated by the Chick-Watson model (Table 7-3);

‡ Synergy was calculated using Equation 3-3.

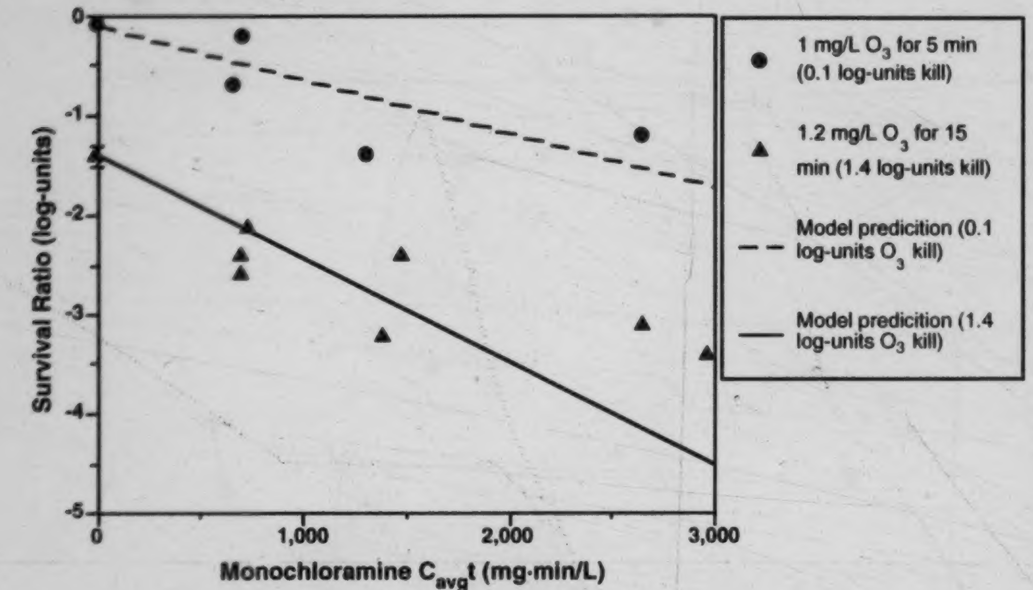


Figure 9-2. Survival ratios of *C. parvum* oocysts as a function of monochloramine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 10°C

monochloramine treatment. For the 1.4 log-units of ozone pre-treatment, the gross kill reached 3 log-units of inactivation when the monochloramine $C_{avg}t$ product was 2,500 mg·min/L.

9.3.3 At 22°C

Data for ozone with monochloramine sequential treatment at 22°C at pH 8 are summarized in Table 9-3. Triplicate tests showed that the ozone dose of 0.4 mg/L and contact time of 4 min at low level pre-treatment induced a kill of 0.1 to 0.7 log-units, with average of 0.4 log-units. Duplicate tests of the secondary monochloramine treatment using a dose of 3 mg/L, induced gross kills from 2.1 to 2.4 log-units for a contact time of 480 min, and as the contact time reached 960 min, gross kills of 3.2 log-units or higher were observed. More than 1.0 log-unit of synergy was observed for monochloramine $C_{avg}t$ products higher than 552 mg·min/L.

Table 9-3. Summary of *C. parvum* oocyst inactivation using ozone followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 22°C

Trial No.	Primary treatment (ozone)				Secondary treatment (Monochloramine)					Total		
	C_o (mg/L)	C_f (mg/L)	t (min)	Observed I_{r1} (log-units)	C_o (mg/L)	C_f (mg/L)	t (min)	$C_{avg}t$ (mg·min/L)	I_{r2} † (log-units)	Observed I_r (log-units)	Synergy‡ (log-units)	Predicted kill (log-units)
727.2 727.3	0.4	0.3	4	0.1	1.3	1.0	480	552	0.1	1.2	1.0	0.7
749.1 749.2	0.4	0.3	4	0.7	3.0	3.0	480	1440	0.3	2.1	1.1	2.1
726.1 726.2	0.4	0.3	4	0.3	3.4	3.2	480	1580	0.4	2.4	1.7	1.9
749.1 749.3	0.4	0.3	4	0.7	3.0	3.0	960	2870	0.7	3.2	1.8	3.6
726.1 726.3	0.4	0.3	4	0.3	3.4	2.9	960	3020	0.7	> 3.3	> 2.3	3.3
723.2 723.3	0.9	0.3	6	1.9	1.7	1.0	360	486	0.1	2.8	0.8	2.7
748.1 748.2	1.0	0.6	5	1.1	2.9	2.9	240	695	0.2	2.4	1.1	2.3
685.1 685.2	0.8	0.4	5	1.5*	3.1	3.1	240	742	0.2	3.0	> 2.1	2.8
703.1 703.2	0.7	0.1	8	1.2	3.1	3.1	240	742	0.2	> 3.1	> 1.7	2.4
751.1 751.2	1.0	0.6	5	1.1	1.9	1.8	480	888	0.2	3.1	1.8	2.6
723.2 723.4	0.9	0.3	6	1.9	1.7	0.2	960	931	0.2	2.9	0.8	3.4
689.2 689.3	0.9	0.4	5	0.9	1.2	0.9	910	956	0.2	2.4	1.3	2.5
748.1 748.3	1.0	0.6	5	1.1	2.9	2.9	480	1390	0.3	3.7	2.3	3.4
703.1 703.3	0.7	0.1	8	1.2	3.1	3.1	480	1480	0.4	> 3.4	> 1.8	3.7
751.1 751.3	1.0	0.6	5	1.1	1.9	1.7	960	1730	0.4	4.1	2.6	4.0
685.1 685.3	0.8	0.4	5	1.5*	3.1	3.1	960	2970	0.7	> 4.2	> 2.8	6.6
703.1 703.4	0.7	0.1	8	1.2	3.1	3.1	960	2970	0.7	> 4.1	> 2.2	6.2

* Ozone kill estimated by I.g.H. model based on the concentration and contact time (Table 5-4);

† Monochloramine kill was estimated by the Chick-Watson model (Table 7-3);

‡ Synergy was calculated using Equation 3-3.

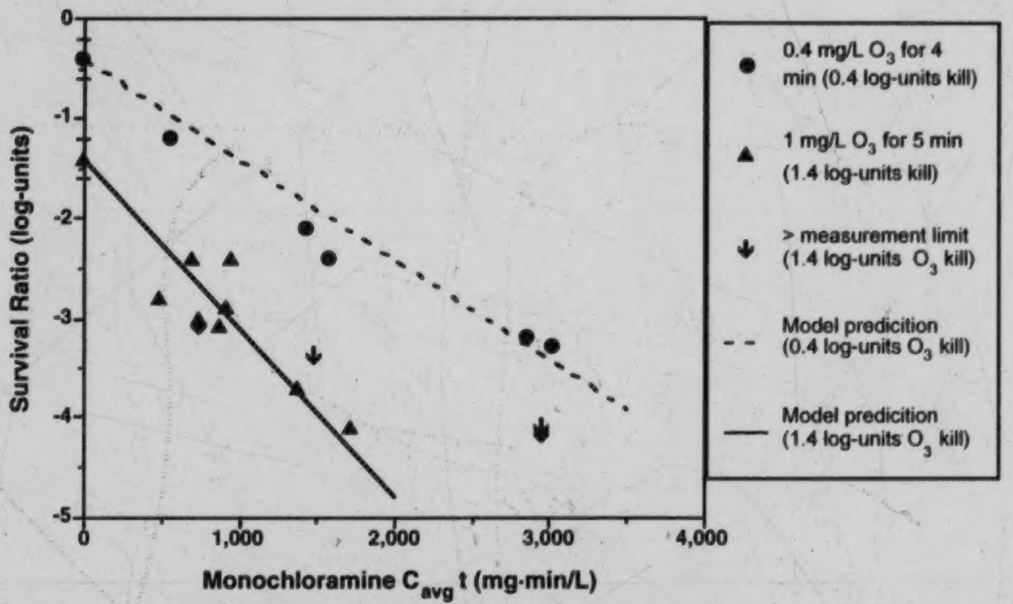


Figure 9-3. Survival ratios of *C. parvum* oocysts as a function of monochloramine $C_{avg}t$ after ozone pre-treatment in oxidant demand-free 0.05 M phosphate buffer at pH 8 at 22°C

For the high level of ozone pre-treatment, an ozone dose of 1 mg/L for 5 min gave a primary inactivation of about 1.4 log-units. In the secondary treatment, the monochloramine dose ranged from 1.2 to 3.1 mg/L with contact time from 240 to 960 min. Evident synergy was observed in all trials, and a consistent synergy of 1.0 log-unit of inactivation was observed for the monochloramine $C_{avg}t$ product higher than 500 mg·min/L.

The relationship of survival ratio after sequential treatment *versus* monochloramine $C_{avg}t$ product is illustrated in Figure 9-3. The oocyst survival curves decreased linearly with the monochloramine $C_{avg}t$ product. For an ozone pre-treatment of 1.4 log-units, the gross kill by the ozone with monochloramine sequential inactivation reached the detection limit of the animal infectivity assay when the monochloramine $C_{avg}t$ product was higher than 1,500 mg·min/L.

9.4 CHICK-WATSON MODELS

From the survival curves of ozone with monochloramine inactivation at different temperatures (Figure 9-4 and Figure 9-5), gross kill of the sequential disinfection increased linearly with the monochloramine $C_{avg}t$ product. A certain level of monochloramine Ct product was required for an evident synergistic effect. For the same level of ozone kill at different temperatures, the slopes of the survival curves were steeper at higher water temperatures. Similar to ozone with free chlorine seqential inactivation, the linear Chick-Watson model (Equation 3-15) was used to estimate the gross kill of ozone with monochloramine sequential inactivation. An individual model was used to predict the gross kill for low or high level of ozone pre-treatment. The effect of temperature on the disinfection rate constant was adjusted using the van't Hoff-Arrhenius relationship. Model parameters and their 90% confidence intervals are listed in Table 9-4. Fitness of observed gross kills and the model predictions are also indicated in Figure 9-4 and Figure 9-5.

Table 9-4. Chick-Watson model parameters for sequential inactivation of *C. parvum* oocysts using ozone followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1 to 22°C

Secondary Chemical	Monochloramine *	
Ozone pre-treatment levels (log-units)	0.4	1.6
Number of data points	12	26
Temperature coefficient $\hat{\theta}$ (90% limits)	1.07 (1.05, 1.09)	1.06 (1.05, 1.07)
$\hat{k}_{22}$ (L/(mg·min)) (90% limits)	0.0010 (0.0009, 0.0011)	0.0017 (0.0015, 0.0018)
Error σ	0.33	0.44
Model constraints for secondary disinfection Initial residual C_o (mg/L) Contact time t (min)	$1.0 \leq C_o \leq 3.4$ $240 \leq t \leq 960$	$0.2 \leq C_o \leq 3.6$ $120 \leq t \leq 960$

* Kinetic model see Equation 3-15

9.5 EFFECT OF OZONE PRE-TREATMENT

The ozone pre-treatment significantly enhanced the inactivation constant of the secondary monochloramine treatment. At the low level of ozone pre-treatment (0.4 log-units), the monochloramine inactivation rate constant was 4 times of that for

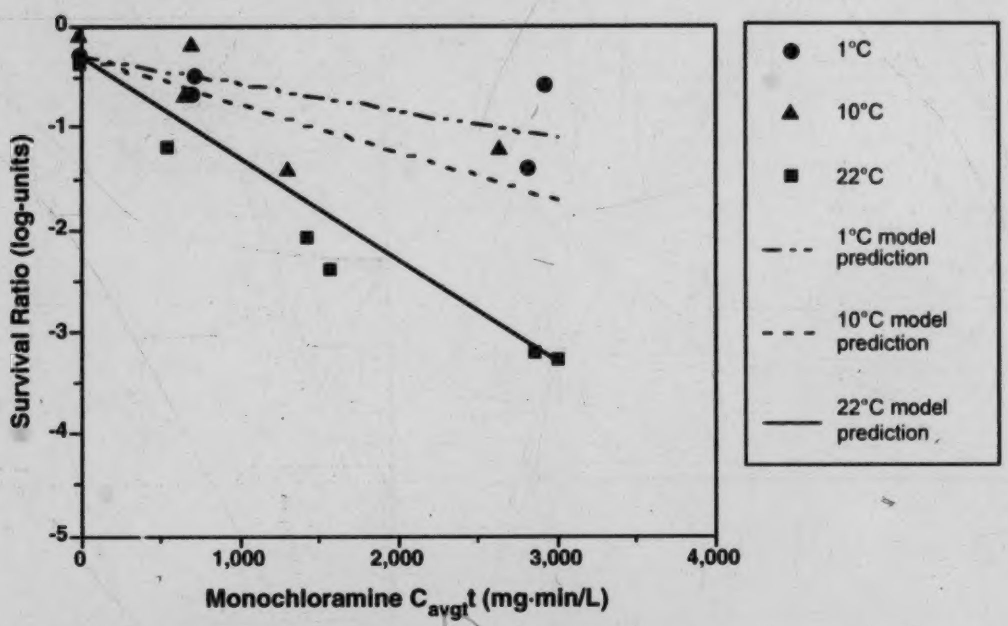


Figure 9-4. Chick-Watson model prediction on inactivation of *C. parvum* oocysts using 0.4 log-units of ozone pre-treatment kill followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1, 10 and 22°C

monochloramine used singly ($\hat{k}_{22}$=0.0010 L/(mg·min) with ozone pre-treatment and $\hat{k}_{22}$=0.00024 L/(mg·min) without ozone pre-treatment). At the high level of ozone pre-treatment, the inactivation rate constant for monochloramine was 7 times that of monochloramine alone ($\hat{k}_{22}$=0.0017 L/(mg·min) with 1.4 log-units of ozone pre-treatment).

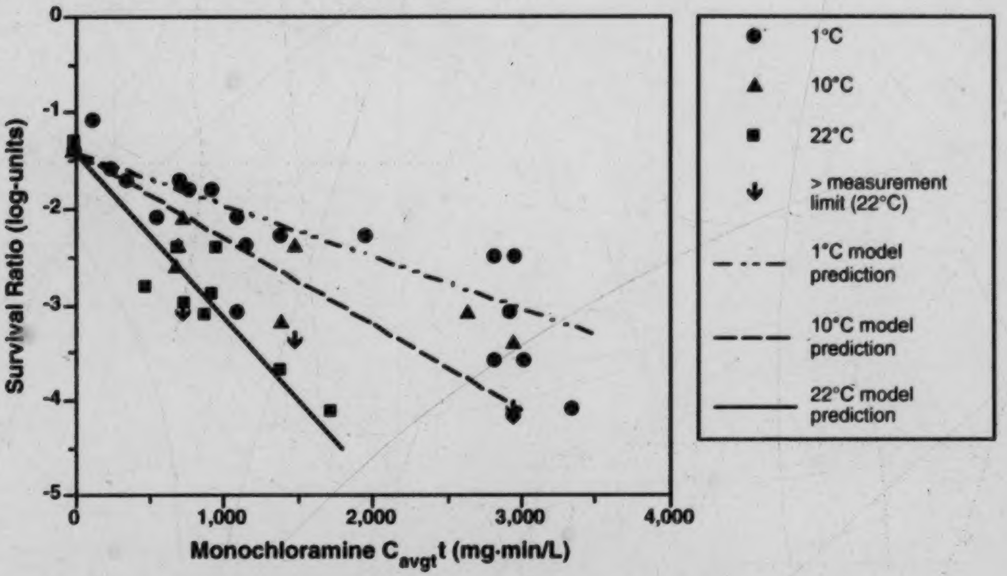


Figure 9-5. Chick-Watson model prediction on inactivation of *C. parvum* oocysts using 1.4 log-units of ozone pre-treatment kill followed by monochloramine in oxidant demand-free 0.05 M phosphate buffer at pH 8 and 1, 10 and 22°C

Comparing the two levels of ozone pre-treatment, the inactivation rate constant for monochloramine secondary treatment after 1.4 log-units of ozone primary inactivation was 70% higher than that for 0.4 log-units of ozone primary inactivation. Thus, a higher level of ozone pre-treatment resulted in a higher gross kill and higher synergy.

The microorganism reduction rate constant for monochloramine secondary treatment after 1.4 log-units of ozone primary inactivation was 70% higher than that for 0.4 log-units of ozone primary inactivation. Thus, a higher level of ozone pre-treatment resulted in a higher gross kill and higher synergy. A previous study (Gyürék et al. 1997) on free chlorine following by monochloramine inactivation of *C. parvum* oocysts showed that increasing levels of free chlorine pre-treatment reduced the subsequent monochloramine Ct requirement for a given level of inactivation.

9.6 EFFECT OF TEMPERATURE

Figure 9-4 and Figure 9-5 show the gross kill *versus* the secondary disinfectant $C_{avg}t$ products at different temperatures. For each ozone pre-treatment, high temperature was favorable for both gross kill and synergy. Similar to ozone and free chlorine inactivation, a pronounced temperature effect was observed for monochloramine secondary treatment. For every 10°C decrease in temperature at low level of ozone pre-treatment (0.4 log-units) the reaction rate constant decreased by a factor of 1.9, while for that of high level of ozone pre-treatment (1.4 log-units) the reaction rate constant decreased by a factor of 1.7. The activation energies were 43 and 36 kJ/mol for low and high level of ozone pre-treatment, respectively. For both free chlorine or monochloramine secondary treatment, similar phenomena were observed that, with lower level of ozone pre-treatment, temperature had stronger effect on the secondary treatment.

9.7 DISCUSSION

Comparing the result for ozone to that of free chlorine and ozone and monochloramine sequential treatments at 22°C, the effect of free chlorine and monochloramine in the secondary treatment was similar when the ozone pre-treatment provided 1.6 log-units of primary inactivation. However, for 0.4 log-units of ozone primary inactivation, greater synergy was observed when free chlorine was used as the secondary disinfectant. It seems that the extent of sub-lethal injury that could cause synergy also depends on the oxidant used in the secondary treatment. A stronger oxidant in the secondary treatment requires a relative minor injury for synergy.

It is known that the combination of monochloramine with other disinfectants increased the efficacy of monochloramine. Berman et al. (1992) reported that free chlorine following by monochloramine was more effective in the inactivation of MS-2 coliphage when compared to adding chlorine or monochloramine singly. Gyürék et al. (1997) reported that the combination of free chlorine and preformed monochloramine could achieve evident synergy in the inactivation of *C. parvum* oocysts. Straub et al. (1995) studied the use of monochloramine and copper in the form of cupric chloride in the inactivation of *E. coli* and MS-2 coliphage, reported synergism for both organisms using the combined chloramine and copper. They reported than 6 log-units of reduction in *E. coli* was observed after an exposure of 2.5 mg/L monochloramine and 0.8 or 0.4 mg/L cupric chloride for 20 min, while for the same level of inactivation using monochloramine alone required a dose of 5 mg/L for 60 min.

9.8 SUMMARY

The sequential inactivation using ozone followed by monochloramine was very effective for *C. parvum* oocyst inactivation. The gross kill for ozone with monochloramine sequential treatment increases approximately linearly with the free chlorine Ct product. A certain level of Ct product for the secondary disinfectant was essential for expression of synergy.

Water temperature was a critical factor for ozone with monochloramine sequential inactivation. Higher water temperature resulted in a higher gross kill and higher synergistic effect. For the 1.4 log-units of ozone primary inactivation, a 10°C decrease of the water temperature resulted in a decrease of the inactivation rate constant by a factor of 1.7.

Ozone pre-treatment greatly enhanced the effect of monochloramine inactivation. In contrast to monochloramine use alone, the monochloramine reaction rate constants after ozone pre-treatment increased 5 and 7 times for 0.4 and 1.6 log-units of ozone primary kills, respectively. Higher ozone pre-treatment resulted in higher gross kill and synergy. For an ozone primary kill of 1.4 log-units at pH 8, the monochloramine $C_{avg}t$ product required for 3.0 log-units gross kill were about 1,000, 1,800 and 3,000 mg·min/L for 22°C, 10°C and 1°C, respectively.

CHAPTER 10

GENERAL DISCUSSION

10.1 SUMMARY OF THE FINDINGS

The final Interim Enhanced Surface Water Treatment Rule (IESWTR) (U.S. Environmental Protection Agency 1998b) regulated the maximum contaminant level goal (MCLG) of zero for *Cryptosporidium* spp. *Cryptosporidium* species are widely dispersed in the aquatic environment (Ahmad et al. 1997) and oocysts can survive in the environment for at least 12 weeks (Olson et al. 1999). Up to 87% of the raw water locations sampled in the U.S.A and Canada contained *Cryptosporidium* spp. (LeChevallier et al. 1991b; Wallis et al. 1996), and about 12% of the groundwater in the U.S.A were positive of *Cryptosporidium* spp. or *Giardia* spp. (Hancock et al. 1998). The multiple barriers approach using watershed protection, pre-treatment, filtration, and disinfection will be required to prevent transmission of *C. parvum* in water supplies. Given the size of *C. parvum* oocysts, 2 to 5 µm in diameter (Soave and Armstrong 1986), *C. parvum* oocysts may breach treatment filters making chemical inactivation the last barrier against transmission of this human pathogen through a water supply.

Conventional chlorination was shown not to be effective for inactivation of *C. parvum* oocysts (Smith et al. 1988). Alternative chemical disinfectants are required for protection of people in the communities against waterborne cryptosporidiosis. In this study, the performance of different chemical disinfectants for inactivation of *C. parvum* oocysts was examined. Inactivation potentials of ozone, chlorine dioxide, chlorine and monochloramine were examined for different temperature and pH conditions. Inactivation kinetic models for each of those oxidants were developed. Sequential inactivation using ozone and chlorine species was also examined using different levels of ozone pre-treatment, temperatures and Ct products of the secondary treatment. Empirical models were developed for sequential inactivation using ozone and chlorine species.

The superior efficacy of ozone has made it one of the leading chemical disinfectants for controlling *C. parvum* in drinking water (Peeters et al. 1989; Korich et al. 1990b; Gyürék et al. 1999). At room temperature, a 3 log-units of inactivation required a Ct product of about 8 mg·min/L, which was practical for water treatment industry with an ozone contact time about 10 min. Chlorine dioxide was an effective oxidant for *C. parvum* inactivation (Peeters et al. 1989; Korich et al. 1990b; Ruffell et al. 2000). A Ct product of 100 mg·min/L was estimated from this study for a 2 log-units of inactivation of *C. parvum* oocysts. To avoid using a high chlorine dioxide dose, a longer contact time was required for the chlorine dioxide process to achieve a 2 log-units of inactivation. Results from this study also confirmed that free chlorine or monochloramine alone were not very effective for *C. parvum* inactivation. A half log-unit of inactivation at room temperature required a $C_{avg}t$ product of 1,500 or 2,000 mg·min/L for hypochlorous acid or monochloramine, respectively. A possible approach to achieve such high Ct values could be maintaining the disinfectant residual in the water reservoir or distribution systems for longer time.

Temperature was critical for chemical microorganism reduction using ozone, chlorine dioxide and chlorine species. At low temperature (between 1 and 22°C), the efficacy of ozone and chlorine dioxide decreased by a factor or 2.1 and 2.3 for each 10°C decrease, respectively. For most water utilities in U.S.A and Canada, winter or spring would be the challenge seasons for the chemical disinfection processes due to low water temperature. Winter and spring are also the peak seasons for the occurrence of *C. parvum* in surface water (Smith et al. 1991; Ketelaars et al. 1995). Thus, actions must be taken to strengthen the filtration and increase the disinfection Ct value to ensure the safety of water in those seasons.

Although pH did not have a direct effect on the efficacy of ozone or chlorine dioxide inactivation of *C. parvum* oocysts, it affected the microbial reduction processes by increasing the decay rate of chemicals at higher pH, especially for ozone. For high alkalinity water, higher ozone doses will be needed to meet the required Ct products. Chlorine dioxide was relatively stable, although a slightly higher decay occurred at high pH (Hoigné and Bader 1994). Water pH also affected the free chlorine inactivation by changing the ratio of hypochlorous acid and hypochlorite ion. At 22°C, hypochlorous acid, the active component for *C. parvum* oocyst inactivation, constituted 97.5, 79.3, and 27.7% of the free chlorine at pH 6, 7, and 8, respectively (White 1992). For the microorganism reduction process using free chlorine, lower pH required a lower chlorine dose to meet the required hypochlorous acid Ct.

Free chlorine or monochloramine is frequently added as the final chemical treatment in water treatment facilities that use ozone as the primary disinfectant. In this study, significant synergistic effect was observed for the sequential inactivation under the practical Ct conditions. For the doses investigated (less than 3.9 mg/L), the gross kill of the sequential inactivation increased linearly with the $C_{avg}t$ products of the secondary disinfectants. Maintaining a certain concentration of free chlorine or monochloramine secondary treatment chemical for an appropriate contact time was essential to achieving synergy. To obtain higher levels of gross kill, higher levels of ozone pre-treatment were necessary. Water temperature also played a key role in sequential inactivation using ozone followed by chlorine species. Higher Ct values for both ozone primary inactivation and free chlorine or monochloramine secondary treatment were required to meet the same target inactivation at lower temperatures.

The synergistic effect of the sequential inactivation compensated some reduction of ozone inactivation at low temperature. Provided an ozone pre-treatment of 1.6 log-units kill at pH 6, a gross kill of 3 log-units required the free chlorine $C_{avg}t$ products of 1,000, 2,000 and 3,500 mg·min/L for 22, 10 and 1°C, respectively. Provided an ozone pre-treatment of 1.6 log-units inactivation and water temperature higher than 10°C, a water disinfection system with a chlorine dose of 2 mg/L and contact time approaching 1,000 min can provide 3 log-units of *C. parvum* oocyst inactivation. The effect of ozone with monochloramine sequential inactivation was comparable to that of the ozone with free chlorine system. Given an ozone pre-treatment causing 1.6 log-units of inactivation at pH 8, a gross kill for 3 log-units required the monochloramine $C_{avg}t$ products of 1,000, 1,800 and 3,000 mg·min/L for 22, 10 and 1°C, respectively, assuming buffered, oxidant demand-free conditions.

10.2 APPLICATION TO NATURAL WATER

The study conducted in oxidant demand-free laboratory water provides uniform, reproducible data. However, disinfectant demand-free conditions are unlikely for most surface and ground waters that contain different organic or inorganic matter (Hoff 1987). To apply the design criteria for the assessment of full scale disinfection processes, the following issues must be resolved: 1) validation of disinfection kinetics in natural water; 2) accounting for the gas-liquid mass transfer efficiency and disinfectant decay; and 3) accounting for the reactor hydraulics.

There are several factors that may affect the inactivation kinetics of *C. parvum* in natural water. Oocysts in natural waters come from different hosts, consequently, their variable physiological state may influence their susceptibility to chemical inactivation. It was reported by Berg et al. (1982), Stewart and Olson (1992b; 1992a) that bacteria grown at sub-maximal rates were more resistant to chemical inactivation than their counterparts grown at maximal rate. Genetic heterogeneity also causes a more significant tail effect (Cerf 1977).

Due to the experimental limitations, the oocyst densities in the reactor were up to 10^8 oocysts/L. Typically, the oocysts detected in surface and groundwater are very few, less than 1 oocyst/L (Lisle and Rose 1995). Only in some cases might the oocyst density reach 6×10^3 oocysts/L (Madore et al. 1987). The differences in oocysts density may also affect the inactivation kinetics.

Adsorption of particles in natural water may change the surface properties of the oocysts. Oocysts Zeta potential is about to -25 mV at pH 6 to 6.5 (Drozd and Schwartzbrod 1996), indicating that they will adsorb particles in the water. Stagg et al. (1978) reported that solids associated with coliphages tended to adsorb to the solid surface, causing the coliphages to be more resistant to chlorine inactivation. Studies on the effect of particulates on ozone disinfection of bacteria and viruses showed that fecal material provided good protection for all these organisms (Sproul et al. 1979). Water with high turbidity might cause the aggregation and mucoid coating of the microorganisms, which added to their resistance to microorganism reduction (Clark et al. 1994). Clumping also increased the required Ct for *Naegleria gruberi* cyst inactivation using chlorine dioxide (Chen et al. 1985). For all of these reasons, *in-situ* bench scale tests are a minimum requirement to validate the model parameters for specific conditions in applying the design criteria to natural waters.

The second issue that should be noted is the oxidant demand and decay in the natural water conditions. High alkalinity, organic content and other water parameters may have direct effects on the initial demand and the decay rate of the oxidants. To ensure the safety, the Surface Water Treatment Rule (SWTR) assumed the effluent concentration as the characteristic disinfectant concentration for the reactor (Malcolm Pirnie Inc. and HDR Engineering Inc. 1991; Lev and Regli 1992a). This approach given by the SWTR can lead to over design of microorganism reduction systems. A rational approach for regulatory design criteria would be to use the temperature corrected I.g.H. or Chick-Watson models that account for the disinfectant decay for a given level of inactivation and set of environmental conditions. These models offer accurate initial disinfectant residual and contact time settings which can be graphically presented as a process design chart. Initial demand of the oxidant can be tested so the dose required for the target inactivation can be calculated. For the dual-phase reactor, the distribution pattern of disinfectants can be predicted using gas-liquid mass transfer models along with the auto-decay term. The model of oxidant concentration as a function of the retention time could be incorporated into the kinetic models to account for its effect on inactivation.

To account for the variance in the experimental data, safety factors (1.5 to 2.0) were applied when SWTR regulated the Ct requirement for *Giardia* spp. inactivation using ozone, chlorine and chlorine species (Malcolm Pirnie Inc. and HDR Engineering Inc. 1991). Adding a safety factor causes the over design of the reactor but improve the confidence level in the design. The concentration and contact time to ensure (1-α)% confidence level should be determined using reverse confidence interval of the model prediction, which was discussed in chapters 3, 5 and 6.

In addition to the inactivation rate law and disinfectant residual concentration, the third issue regarding the design or assessment of disinfection contactors is reactor hydraulics. A simple approach for accounting for reactor hydraulics was that given by SWTR using t_{10} as the characteristic contact time for non-ideal reactor flow conditions (Malcolm Pirnie Inc. and HDR Engineering Inc. 1991; Lev and Regli 1992b). To better account for the reactor hydraulics, the temperature corrected Hom-type models and Chick-Watson type models applied in this study can be coupled with hydraulic dispersion models to account for non-ideal hydraulics. The N-CFSTR or axial dispersion model (Roustan et al. 1995) may be used in the simulation. *In-situ* pilot scale experiments would be ideal to validate reactor hydraulics and the gas-liquid mass transfer and decay of the disinfectants.

CHAPTER 11

CONCLUSIONS AND RECOMMENDATIONS

11.1 CONCLUSIONS

From the study of *C. parvum* oocyst inactivation using single or combined microorganism reduction chemicals in buffered high purity water, the following conclusions have been reached:

1. Ozone is the most effective single chemical for the inactivation of *C. parvum* oocysts. At room temperature, a 3.0 log-units inactivation of oocysts was practical for ozone treatment. Oocyst survival curves under ozone treatment were characterized by an initial lag and an evident tail-off, which were more evident at low temperature. The non-linear temperature corrected I.g.H. model gave an adequate description of ozone inactivation kinetics. Water temperature was a critical factor in ozone inactivation, while pH was not as important within pH 6 to 8. Within 1 to 37°C, temperature effect on ozone inactivation rate was described by the van't Hoff-Arrhenius relationship. For every 10°C temperature decrease, the I.g.H. model reaction rate constant decreased by a factor of 2.2, corresponding to an activation energy of 52 kJ/mol.

2. Chlorine dioxide is very effective for the inactivation of *C. parvum*

oocysts. Oocysts survival curves under chlorine dioxide treatment decreased linearly with the $C_{avg}t$ products. Temperature was a critical factor for chlorine dioxide inactivation, while pH was not statistically important to the inactivation rate constant within the pH range of 6 to 11. The Chick-Watson model with temperature correction was suitable for predicting of chlorine dioxide inactivation at 1 to 37°C and pH 6 to 11. For every 10°C temperature decrease, the model reaction rate constant decreased by a factor of 2.3, corresponding to an activation energy of 55 kJ/mol.

3. Free chlorine or monochloramine has little effect on *C. parvum* oocyst viability under the practical doses and contact times. The temperature corrected $C_{avg}t$ models were adequate for description of free chlorine or monochloramine inactivation of *C. parvum* oocysts. At 22°C, free chlorine was about 50% more effective than monochloramine. Temperature was a significant factor for free chlorine or monochloramine inactivation. For every 10°C decrease in temperature, the inactivation rate decreased by a factor of 2.3 and 2.6 for free chlorine and monochloramine, respectively.

4. Significant synergy was observed for ozone followed by free chlorine treatment of *C. parvum* oocysts at pH 6. The level or synergy was related to the level of ozone pre-treatment, Ct products of free chlorine, and water

temperature. The gross kill and synergistic effect increased linearly with the $C_{avg}t$ products of the free chlorine secondary treatment. The higher level of ozone pre-treatment resulted in a higher gross kill. Higher water temperature was favorable to both gross kill and synergy. The gross kills for ozone with free chlorine sequential inactivation were predicted using an empirical Chick-Watson typed model with a temperature correction. For about 1.6 log-units of ozone pre-treatment, the free chlorine $C_{avg}t$ products required for 1.0 log-units of synergy were 700, 1,500 and 2,500 mg·min/L for 22, 10 and 1°C, respectively.

5. Significant synergy was observed for ozone followed by monochloramine sequential inactivation of *C. parvum* oocysts. The level or synergistic effect was related to the level of ozone pre-treatment, $C_{avg}t$ products of the secondary treatment, and water temperature. The levels of gross kill and synergistic effect increased linearly with the $C_{avg}t$ products of the monochloramine secondary treatment. High water temperature was favorable to both gross kill and synergy. The gross kill of ozone with monochloramine sequential inactivation was predicted using an empirical Chick-Watson typed model with temperature correction. For an ozone pre-treatment induced 1.4 log-units of inactivation, the monochloramine $C_{avg}t$ products required for 1.0 log-units of synergy were 500, 1,500 and 2,500 mg·min/L for 22, 10 and 1°C, respectively.

11.2 RECOMMENDATIONS

Despite the important findings reported in this study, several issues remain to be addressed before applying the inactivation requirement for *C. parvum* oocysts using single or combined chemical disinfectants. It is recommended that the following issues should be studied:

1. The influence of water characteristics such as turbidity, organic or inorganic compounds in the inactivation kinetics of single and sequential inactivation;
2. The mechanism of synergy and the approach to optimize the synergistic effect in the sequential inactivation;
3. The effect of growth condition and the environmental exposure on *C. parvum* oocyst susceptibility to the single or sequential chemical treatments;
4. The approach to convert the inactivation requirement from the laboratory results to the natural conditions based on the water characteristics; and
5. The possibility of applying the Ct requirement estimated from the batch reactor to the continous flow systems using natural water.

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APPENDIX A

CONCENTRATION AND CONTACT TIME PROFILE FOR EXPERIMENTAL TRIALS

When ozone or chlorine dioxide were used as the oxidants, the oxidant residual concentration during the contact time period was monitored continuously. Figure A-1 shows an example of the concentration and contact time profile (Trial No. 611). The applied ozone dose was 1 mg/L and contact time was 10 min. The initial ozone demand was about 0.1 mg/L. The initial baseline absorbance was equal to an ozone concentration of about 0.19 mg/L. After the ozone treatment, the baseline was slightly lowered because some UV absorbed components had been oxidized by ozone.

Other information for Trial no. 611 are listed as follow:

Solutions	oxidant demand-free 0.05 phosphate buffer (200 mL solution in 250 mL reactor)
pH	8.0
Temperature	22°C
Number of oocysts	5×10^7
Applied ozone	1.0 mg/L
Contact time	$t = 10$ min
Initial ozone residual	$C_o = 0.9$ mg/L
Final ozone residual	$C_f = 0.6$ mg/L
First order ozone decay	$k' = 0.04\ \text{min}^{-1}$

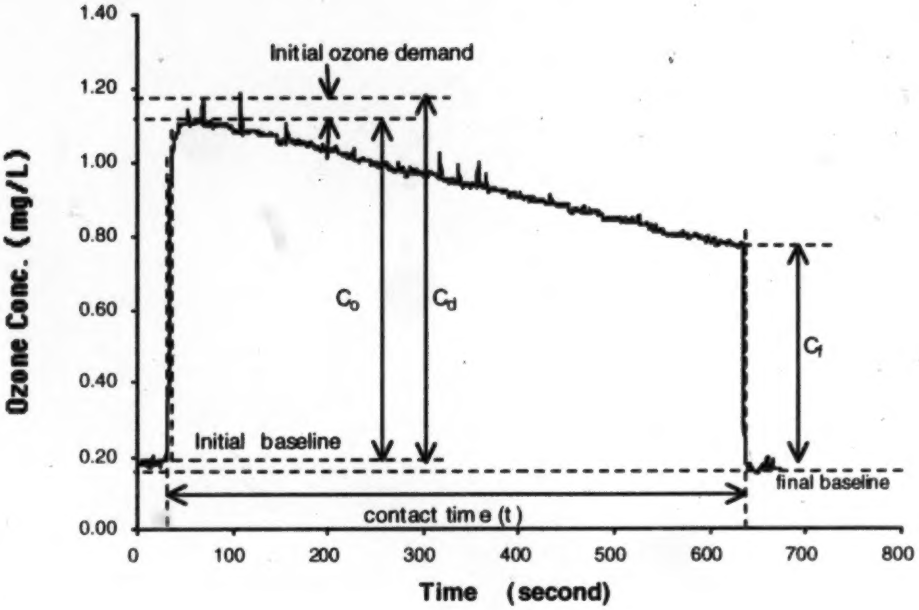

Figure A-1. Ozone residual concentration *versus* time profile for trial No. 611 (pH 8.0 and 22°C)

APPENDIX B

DOSE-RESPONSE DATA

Table B-1. Dose-response data of neonatal CD-1 mouse to *C. parvum* oocysts (batch # 1 to 7)

Batch 1		Batch 2		Batch 3		Batch 4		Batch 5		Batch 6		Batch 7	
X	P	X	P	X	P	X	P	X	P	X	P	X	P
18	0/9	10	2/20	50	5/25	25	0/6	35	6/17	10	1/33	35	3/10
25	2/16	20	8/20	100	6/20	50	5/28	70	10/20	20	2/25	50	5/20
40	3/11	40	5/30	200	11/15	75	2/20	140	15/19	40	9/25	70	1/20
43	1/5	60	10/20	300	13/14	100	8/40			80	13/23	100	3/10
50	1/14	65	5/10	400	20/25	162	4/12			160	28/33	140	5/10
53	1/4	67	4/5	500	8/10	188	3/7					200	14/20
73	5/7	70	1/5	800	11/14	194	2/10					280	5/10
75	9/13	80	15/20	1600	8/10	200	11/17					400	18/20
100	4/23	97	6/10			225	2/14						
106	4/4	100	10/18			281	0/6						
109	5/5	670	3/4			300	5/5						
125	5/5	700	3/5			331	1/6						
133	7/7	6700	5/5			352	3/5						
150	3/8	7000	5/5			400	10/20						
156	6/9					412	8/10						
183	4/6					600	3/5						
197	6/6					664	4/5						
200	16/24					800	6/10						
225	8/8												
250	6/6												
300	8/8												
306	6/9												
328	5/5												
400	11/11												

Note: X = inoculum (number of oocysts given to each CD-1 mouse);

P = N_i/N_m, where N_i = number of mice scoring positive for infection and N_m = number of mice in the cohort.

TABLE B-2. Dose-response data of neonatal CD-1 mouse to *C. parvum* oocysts (batch # 8 to 14)

Batch 8		Batch 9		Batch 10		Batch 11		Batch 12		Batch 13		Batch 14	
X	P	X	P	X	P	X	P	X	P	X	P	X	P
50	2/20	20	1/6	25	6/30	25	0/7	50	3/10	50	9/20	35	2/10
70	0/20	25	9/28	35	7/17	35	9/37	100	8/10	100	11/20	50	4/10
100	8/30	40	8/20	50	10/27	50	4/9	200	5/10	200	16/20	70	6/10
140	5/20	50	18/25	70	22/39	70	24/53	400	9/10	400	15/20	100	7/10
200	12/30	80	15/20	100	21/30	100	3/5					140	7/10
280	13/20	100	32/39	140	35/38	140	47/66					200	9/10
400	14/20	160	5/5	200	23/28	280	52/59					280	10/10
560	16/20	200	33/33	280	28/30	540	7/7					400	9/10
800	9/10	800	4/4										

Note: X = inoculum (number of oocysts given to each CD-1 mouse);

P = N_i/N_m, where N_i = number of mice scoring positive for infection and N_m = number of mice in the cohort.

Table B-3. Dose-response data of neonatal CD-1 mouse to *C. parvum* oocysts (batch # 15 to 19)

Batch15		Batch 16		Batch17		Batch 18		Batch 19	
X	P	X	P	X	P	X	P	X	P
20	0/10	40	3/20	20	2/10	25	11/40	25	2/40
40	7/20	80	9/20	40	4/20	50	15/30	50	16/60
50	5/10	160	15/20	50	1/5	100	29/40	100	33/55
80	11/20	320	18/20	80	7/20	200	43/55	200	44/50
160	11/20			100	2/5	400	10/10		
200	9/10			160	7/10				
240	9/10			200	3/5				
320	7/10			320	15/20				
				400	4/5				
				640	10/10				

Note: X = inoculum (number of oocysts given to each CD-1 mouse);

P = N_i/N_m, where N_i = number of mice scoring positive for infection and N_m = number of mice in the cohort.

APPENDIX C

SUMMARY OF CONTROL TRIALS

Table C-1. Control trials of *C. parvum* inactivation tests in oxidant demand-free 0.05 M phosphate buffer solution

Trial No.	Temp. (°C)	pH	Bath oocyst batch No.	Buffer volume (mL)	Quenching reagent	Quenching reagent Volume (mL)	Observed kill (log-units)[a]
700	1	6	17	200	F	0.4	-0.8
495	1	6	6	200	T	1	-0.3
733.1	1	6	17	200	S	0.2	-0.2
709	1	6	17	200	F	0.4	-0.2
636	1	6	8	400	S	0.4	-0.1
501	1	6	13	200	F	0.4	0.4
591	1	6	6	200	T	1	0.4
657	1	6	14	200	S	0.4	0.5
635	1	6	8	400	S	0.4	0.9
546	1	7	7	200	F	0.4	-0.3
682	1	8	17	400	S	0.4	-0.8
687.1	1	8	17	200	S	0.4	-0.8
702	1	8	17	200	F	0.4	-0.5
721.1	1	8	17	200	F	0.4	-0.5
720.1	1	8	17	200	F	0.4	-0.2
688.1	1	8	17	200	F	0.4	-0.2
674	1	8	16	200	F	0.4	0.1
560	1	8	17	200	F	0.4	0.1
724.1	1	8	7	200	S	0.4	0.1
519	1	8	6	200	S	0.4	0.2
531	1	8	17	200	S	0.2	0.3
676	1	8	16	200	S	0.4	0.3
745.1	1	8	8	400	S	0.4	0.3
633	1	8	8	400	S	0.4	0.3
634	1	8	6	200	T	1	0.3
585	1	11	13	200	T	1	0.9
539	5	7	12	200	F	0.4	-0.2
535	5	7	12	200	F	0.4	0.1
568.1	5	7	13	200	F	0.4	0.7

(continued)

Table C-1 (Continued)

Trial No.	Temp. (°C)	pH	Bath oocyst batch No.	Buffer volume (mL)	Quenching reagent	Quenching reagent Volume (mL)	Observed kill (log-units)
568.2	5	7	13	200	S	0.4	0.7
691	5	7	17	80	F	0.16	-0.2
693	5	7	17	80	F	0.16	-0.2
750.1	10	6	17	200	S	0.2	-0.2
705	10	8	17	200	F	0.4	-0.7
684	10	8	17	200	F	0.4	0.1
728.1	10	8	17	400	S	0.4	0.1
743.1	10	8	17	200	S	0.2	0.5
571.1	13	7	13	200	S	0.4	-0.3
613	20	7	8	200	T	1	0.0
615	20	7	8	200	T	1	0.8
729.1	22	6	17	200	S	0.2	-0.2
718.1	22	6	17	200	F	0.4	0.0
641	22	6	17	200	S	0.2	0.1
719.1	22	6	17	200	S	0.2	0.1
737.1	22	6	17	200	F	0.4	0.1
741.1	22	6	8	200	S	0.4	0.1
511	22	7	6	200	T	1	0.2
521	22	7	6	200	F	0.4	0.4
523	22	7	6	200	F	0.4	0.5
620	22	7	8	200	T	0.4	0.0
622	22	7	8	200	T	0.4	0.1
685	22	8	17	400	S	0.4	-0.8
703	22	8	17	200	F	0.4	-0.5
727.1	22	8	17	200	F	0.4	-0.5
689.1	22	8	17	200	F	0.4	-0.2
723.1	22	8	17	200	F	0.4	0.1
643	22	8	8	200	F	1	0.3
639	22	11	8	200	S	0.4	0.2
Summary			Mean kill (log-units)				0.03
			Std. Deviation (log-units) (n=58)				σ = 0.42

Note: Quenching reagent: F= 1 M sodium formate; S = 1 M sodium sulfite; and T = 0.1 M sodium thiosulfate

APPENDIX D

ANIMAL DATA FROM EXPERIMENTAL AND CONTROL TRIALS

Table D-1. Animal data of viability assay using neonatal CD-1 mouse for experimental and control trials

Trial No.	Batch No.	Inoculum (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log units)
		A	B	A	B	A	B	
35	2	20	42	5	5	1	2	0.1
37	2	20	40	5	5	1	2	0.1
42	2	100	1,000	5	5	1	1	1.0
55	2	60	598	5	5	1	5	0.3
57	2	100	1,004	5	5	0	5	1.4
72	2	55	547	4	5	0	5	0.1
73	2	469	4,687	5	5	1	1	1.6
74	2	52	519	5	5	1	3	0.6
102	2	500	5,000	5	4	3	4	0.6
141	3	6,000	60,000	5	5	4	5	1.2
148	3	800	8,000	5	5	4	5	0.2
174	3	200	2,000	5	4	4	4	-0.4
210	4	1,500	5,000	5	5	0	3	1.0
218	4	15,000	150,000	5	5	0	4	2.1
249	9	150	1,500	4	5	2	5	0.3
265	9	40	400	5	5	1	5	0.3
267	9	30	300	5	5	1	5	0.2
268	9	250	2,500	5	5	2	5	0.9
270	9	100	1,000	4	5	3	5	0.1
370	10	40	400	7	7	3	5	0.3
372	10	40	400	10	10	6	10	-0.3
373	10	400,000	1,600,000	9	8	8	8	3.3
374	11	400	4,000	9	8	0	3	2.0
376	10	40	400	10	10	7	9	0.0
380	10	1,200	12,000	10	10	0	3	2.5
382	10	1,400	14,000	10	10	1	9	2.0
437	10	400	4,000	8	8	3	8	1.3
439	11	40	400	10	8	5	7	-0.1

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
446	11	200	2,000	5	5	0	1	2.8
448	11	200	2,000	7	8	1	2	1.1
494	6	150	1,500	10	10	4	10	0.5
495	6	40	400	10	10	6	10	-0.3
496	6	40	400	10	10	3	10	0.0
497	6	50,000	500,000	10	10	5	10	2.9
498	6	2,000	20,000	9	10	7	10	1.2
500	6	150	1,500	8	8	1	6	1.0
501	6	70	700	8	8	3	7	0.4
506	6	100	1,000	8	7	2	7	0.5
507	6	7,000	70,000	8	8	4	8	2.0
511	6	60	600	10	10	3	10	0.2
519	6	40	400	10	10	5	7	0.2
521	6	60	600	8	8	3	6	0.4
523	6	60	600	8	8	1	7	0.5
528	6	6,000	60,000	8	8	4	7	2.2
529	6	70,000	700,000	8	8	6	8	2.8
531	6	40	400	10	10	0	10	0.3
534	12	20,000	200,000	10	10	8	10	1.8
535	12	70	700	10	10	5	8	0.1
539	12	70	700	10	10	6	10	-0.2
541	7	2,000	20,000	10	10	0	1	2.9
542	7	300	3,000	10	10	2	7	0.9
543	7	700	7,000	10	10	2	8	1.2
544	7	800	8,000	10	10	0	6	1.6
545	7	20,000	200,000	10	10	3	7	2.7
546	7	50	500	10	10	4	10	-0.3
548	7	1,000	10,000	10	10	4	10	1.0
555	7	80,000	800,000	10	9	1	4	3.7
556	7	80,000	800,000	10	10	3	9	3.1
557	7	50	500	10	10	2	6	0.2

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
558	7	200	2,000	10	10	3	7	0.7
560	7	80	800	10	10	2	9	0.1
563	7	10,000	100,000	10	10	2	3	2.7
564	7	10,000	100,000	10	10	1	7	2.6
566	13	10,000	100,000	10	10	5	9	2.1
567	13	100	1,000	10	10	4	8	0.4
568.1	13	50	500	5	5	0	3	0.7
568.2	13	50	500	5	5	1	3	0.7
568.3	13	100	1,000	5	5	2	5	0.4
568.4	13	500	5,000	5	5	2	5	1.1
568.6	13	5,000	50,000	5	5	2	4	2.1
569	13	10,000	100,000	10	10	2	10	3.0
570	13	2,000	20,000	10	10	3	7	2.0
571.1	13	50	500	7	7	4	7	-0.3
571.2	13	50	500	7	7	4	6	-0.2
571.3	13	500	5,000	7	7	3	6	1.0
571.4	13	1,000	10,000	7	7	0	4	2.0
571.5	13	5,000	50,000	7	7	2	6	2.1
572	13	8,000	80,000	10	10	6	10	1.9
573	13	8,000	80,000	10	10	7	10	1.6
576	13	1,000	10,000	10	10	6	9	1.0
577	13	100	1,000	10	10	1	7	1.1
579	13	10,000	100,000	10	10	0	6	3.0
580	13	10,000	100,000	10	10	3	10	2.7
581	13	100,000	1,000,000	10	10	2	6	4.0
582	13	1,000	10,000	10	10	0	3	2.7
584	13	10,000	100,000	10	10	0	9	1.9
585	13	50	500	7	7	2	2	0.9
586	13	100	1,000	7	7	2	3	1.0
587	13	100	1,000	10	10	5	10	0.2
588	13	100	1,000	10	10	6	9	0.0

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
591	13	50	500	10	10	1	9	0.4
596	8	1,000	10,000	10	10	2	8	1.1
597	8	10,000	100,000	10	10	6	10	1.5
598	8	400	4,000	10	10	5	8	0.6
599	8	10,000	100,000	10	10	4	5	2.2
600	8	30,000	300,000	10	10	5	9	2.3
601	8	50	500	9	10	2	8	-0.2
602	8	50	500	10	10	2	8	-0.2
603	8	500	5,000	9	9	2	7	0.8
604	8	100	1,000	9	9	1	9	0.2
605	8	10,000	100,000	10	10	0	3	2.8
606	8	10,000	100,000	10	10	0	3	2.8
608	8	1,000	10,000	10	10	3	7	1.1
609	8	100	1,000	10	10	4	7	0.1
613	8	50	500	10	10	1	7	0.0
615	8	50	500	8	8	0	1	0.8
617	8	20,000	200,000	10	10	1	8	2.5
618	8	20,000	200,000	8	8	2	4	2.6
620	8	100	1,000	10	10	2	9	0.0
622	8	100	1,000	8	8	2	6	0.1
624.1	8	2,000	20,000	5	5	1	4	1.4
627.1	8	2,000	20,000	5	5	1	3	1.6
627.3	8	400	4,000	5	5	2	5	0.3
627.5	8	400	4,000	5	5	2	4	0.6
628.1	8	400	4,000	7	7	3	7	0.3
628.2	8	400	4,000	7	7	5	7	0.0
631.1	8	2,000	20,000	5	5	3	5	0.8
631.3	8	400	4,000	5	5	2	5	0.3
632.1	8	10,000	-	5	-	3	-	1.5
632.5	8	400	4,000	5	5	4	5	-0.1
633	8	400	4,000	5	5	2	5	0.3

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
634	8	400	4,000	5	5	2	5	0.3
635	8	400	4,000	5	5	0	4	0.9
636	8	400	4,000	5	5	4	5	-0.1
638	8	1,000	10,000	10	10	1	5	1.4
639	8	100	1,000	10	10	3	6	0.2
640	8	1,000	10,000	10	10	0	9	1.1
641	8	100	1,000	10	10	2	8	0.1
643	8	100	1,000	10	10	1	7	0.3
647	14	700	7,000	10	10	6	9	1.2
648	14	700	7,000	10	10	8	10	0.7
652	14	50	500	10	10	1	10	0.4
653	14	50	500	10	10	0	10	0.4
656	14	50	500	10	10	3	9	0.2
657	14	50	500	10	10	2	6	0.5
661.1	15	4,000	40,000	10	10	9	10	0.9
661.3	15	60,000	600,000	10	10	9	10	2.08
662	15	200	2,000	8	8	4	8	0.4
663	15	500	5,000	8	8	3	8	1.0
665.1	15	3,000	30,000	7	7	2	2	2.4
665.2	15	10,000	100,000	7	6	2	4	2.6
665.3	15	50,000	500,000	7	7	1	6	3.3
666.1	15	3,000	30,000	7	7	1	3	2.5
666.2	15	10,000	100,000	7	6	2	5	2.5
666.3	15	20,000	200,000	6	6	3	1	3.2
667	15	200	2,000	10	10	7	9	0.3
668	15	100	1,000	10	10	5	10	0.1
670.1	15	1,000	10,000	7	6	2	4	1.6
671.1	15	400	4,000	7	7	4	7	0.6
671.2	15	400	4,000	6	6	1	6	1.3
671.3	15	1,000	10,000	7	7	3	7	1.2
672.1	15	1,000	10,000	5	5	3	4	1.3

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
674	16	80		6		2		0.1
674.1	16	1,000	10,000	5	5	3	5	0.9
674.2	16	1,000	10,000	6	6	1	3	1.7
674.3	16	4,000	40,000	6	6	1	1	2.5
676	16	80	800	6	5	1	5	0.3
677.1	16	400	4,000	7	6	5	5	0.8
677.2	16	500	5,000	7	6	4	6	0.7
677.3	16	900	9,000	7	7	1	7	1.4
682	17	50	-	5	-	4	-	-0.8
682.1	17	5,000	50,000	6		6		< 1.1
682.3	17	30,000	300,000	6	6	4	5	2.5
683.1	17	500	5,000	7	6	7	6	< 0.0
683.2	17	500	5,000	7	6	4	6	0.5
684	17	50	-	5	-	1	-	0.1
684.1	17	5,000	50,000	6	5	6	5	< 1.1
684.2	17	8,000	80,000	6	6	2	6	2.1
685	17	50	-	5	-	4	-	-0.8
685.1	17	2,000	20,000	6	5	6	5	< 0.7
685.2	17	6,000	60,000	6	6	0	2	3.0
685.3	17	50,000	500,000	6	6	0	0	> 4.2
686.1	17	1,000	10,000	5	5	1	5	1.4
686.2	17	1,000	10,000	5	5	0	3	1.8
686.3	17	5,000	50,000	5	5	0	1	3.1
687.1	17	50	500	5	5	4	5	-0.8
687.2	17	50	500	5	5	3	5	-0.5
688.1	17	50	500	5	5	3	4	-0.2
688.2	17	100	1,000	5	5	3	5	-0.2
688.3	17	300	3,000	5	5	2	5	0.6
689.1	17	50	500	5	5	2	5	-0.2
689.2	17	600	6,000	5	5	2	5	0.9
689.3	17	4,000	40,000	5	5	1	2	2.4

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
691	17	50	500	5	5	2	5	-0.2
693	17	50	500	5	5	2	5	-0.2
699.1	17	5,000	50,000	5	5	3	5	1.5
699.2	17	5,000	50,000	5	5	3	5	1.5
699.3	17	50,000	500,000	5	5	3	5	2.5
700	17	50	-	5	-	4	-	-0.8
700.1	17	100	1,000	5	5	4	5	-0.5
700.2	17	500	-	5	-	4	-	0.2
701.1	17	2,000	20,000	5	5	4	5	0.8
701.2	17	2,000	20,000	5	5	1	5	1.7
701.3	17	5,000	50,000	5	5	2	5	1.8
702	17	50	-	5	-	3	-	-0.5
703	17	50	500	5	5	3	5	-0.5
703.1	17	600	6,000	5	5	1	5	1.2
703.2	17	5,000	50,000	5	5	0	0	> 3.1
703.3	17	10,000	100,000	5	5	0	0	> 3.4
703.4	17	50,000	500,000	5	5	0	0	> 4.1
704.1	17	800	8,000	5	5	2	4	1.2
704.2	17	1,000	10,000	5	5	0	1	2.4
704.3	17	5,000	50,000	5	5	0	1	3.1
705	17	50	-	4	-	3	-	-0.7
705.1	17	100	1,000	5	5	2	5	0.1
705.2	17	500	-	5	-	4	-	0.2
705.3	17	500	5,000	5	5	0	4	1.2
706	17	1,000	10,000	10	10	3	8	1.4
707	17	100	1,000	10	10	4	9	0.1
709	17	100	-	10	-	6	-	-0.2
710.2	17	200	2,000	7	7	2	6	0.6
710.3	17	1,000	10,000	7	7	2	5	1.4
711.1	17	5,000	50,000	7	7	5	7	1.3
711.2	17	30,000	-	7	-	4	-	2.3

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
711.3	17	20,000	200,000	7	7	0	2	3.6
716.1	17	1,000	10,000	5	5	0	3	1.8
716.2	17	5,000	50,000	5	5	3	5	1.5
717.2	17	1,000	10,000	5	5	2	4	1.3
717.3	17	10,000	100,000	5	5	0	1	3.4
718.1	17	50	500	5	5	2	4	0.0
719.1	17	50	500	5	5	0	5	0.1
719.2	17	1,000	10,000	5	5	0	4	1.5
719.3	17	5,000	50,000	5	5	0	0	> 3.1
719.4	17	50,000	500,000	5	5	0	0	> 4.1
720.1	17	50	500	5	5	2	5	-0.2
720.2	17	5,000	50,000	5	5	1	3	2.3
720.5	17	50,000	500,000	5	5	0	1	4.1
721.1	17	50	500	5	5	3	5	-0.5
721.2	17	5,000	50,000	5	5	3	5	1.5
721.3	17	5,000	50,000	5	5	1	5	2.1
721.4	17	10,000	100,000	5	5	0	2	3.1
723.1	17	50	500	5	5	1	4	0.1
723.2	17	1,000	10,000	5	5	1	1	1.9
723.3	17	20,000	200,000	5	5	0	4	2.8
723.4	17	30,000	300,000	5	5	1	4	2.9
724.1	17	50	500	5	5	1	4	0.1
724.2	17	1,000	10,000	5	5	0	5	1.4
724.3	17	2,000	20,000	5	5	3	5	1.1
724.4	17	10,000	100,000	5	5	2	5	2.1
725.1	17	1,000	10,000	5	5	3	2	1.4
725.2	17	2,000	20,000	5	5	2	4	1.6
725.3	17	10,000	100,000	5	5	2	4	2.3
726.1	17	100	1,000	5	5	2	4	0.3
726.2	17	1,000	10,000	5	5	0	1	2.4
726.3	17	8,000	80,000	5	5	0	0	> 3.3

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
727.1	17	50	500	5	5	3	5	-0.5
727.2	17	100	1,000	5	5	2	5	0.1
727.3	17	500	5,000	5	5	0	4	1.2
727.4	17	2,000	20,000	5	5	0	1	2.7
728.1	17	50	500	5	5	1	4	0.1
728.2	17	1,000	10,000	5	5	1	3	1.6
728.3	17	10,000	100,000	5	5	0	5	2.4
728.4	17	10,000	100,000	5	5	0	1	3.4
729.1	17	50	500	5	5	2	5	-0.2
729.2	17	1,000	10,000	5	5	0	4	1.5
729.3	17	10,000	100,000	5	5	3	5	1.8
729.4	17	20,000	200,000	5	5	0	4	2.8
730.1	17	1,000	10,000	5	5	0	4	1.5
730.2	17	10,000	100,000	5	5	0	2	3.1
730.3	17	100,000	1,000,000	5	5	1	1	3.9
731.1	17	1,000	10,000	5	5	1	3	1.6
731.2	17	4,000	40,000	5	5	4	5	1.1
731.3	17	10,000	100,000	5	5	1	5	2.4
732.1	17	1,000	10,000	5	4	0	3	1.6
732.2	17	10,000	100,000	5	5	2	4	2.3
732.3	17	100,000	1,000,000	5	5	1	3	3.6
733.1	17	50	500	5	5	2	5	-0.2
734.1	17	1,000	10,000	5	5	1	1	1.9
734.2	17	20,000	200,000	5	5	2	4	2.6
734.3	17	100,000	1,000,000	5	5	1	4	3.4
736.1	17	1,000	10,000	5	5	2	4	1.3
736.2	17	5,000	50,000	5	5	2	4	2.0
736.3	17	20,000	200,000	5	5	1	4	2.7
737.1	17	50	500	5	5	0	5	0.1
737.2	17	100	1,000	5	5	1	5	0.4
733.2	17	100	1,000	5	5	1	5	0.4

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
733.3	17	1,000	10,000	5	5	1	5	1.4
737.3	17	2,000	20,000	5	5	0	0	> 2.7
737.4	17	100,000	1,000,000	5	5	0	1	4.4
741.1	17	50	500	5	5	0	5	0.1
741.2	17	100	1,000	5	5	2	5	0.1
741.3	17	300	3,000	5	5	0	4	0.9
741.4	17	1,000	10,000	5	5	3	5	0.8
742.1	17	100	1,000	5	5	2	4	0.3
742.2	17	5,000	50,000	5	5	1	3	2.3
742.3	17	20,000	200,000	5	5	0	4	2.8
743.1	17	50	500	4	5	0	3	0.5
743.2	17	100	1,000	5	4	2	4	0.1
743.3	17	600	6,000	4	5	2	5	0.7
743.4	17	4,000	40,000	5	5	3	5	1.4
744.1	17	1,000	10,000	5	5	1	4	1.4
744.2	17	10,000	100,000	5	5	1	3	2.6
744.3	17	20,000	200,000	5	4	0	2	3.2
745.1	17	50	500	5	5	1	3	0.3
745.2	17	100	1,000	5	5	2	5	0.1
745.3	17	100	1,000	5	5	3	5	-0.2
746.1	17	100	1,000	5	5	1	5	0.4
746.2	17	100	1,000	4	5	2	4	0.2
746.3	17	100	1,000	5	5	2	4	0.3
748.1	18	1,000	10,000	5	5	3	5	1.1
748.2	18	20,000	200,000	5	5	3	5	2.4
748.3	18	100,000	1,000,000	5	5	1	4	3.7
749.1	18	100	1,000	5	5	1	5	0.7
749.2	18	5,000	50,000	5	5	2	5	2.1
749.3	18	80,000	800,000	5	5	4	4	3.2
750.1	18	50	500	5	5	3	5	-0.2
750.2	18	100	1,000	5	5	2	5	0.4

(continued)

Table D-1 (continued)

Trial No.	Batch No.	Inoculum X (oocysts/animal)		Cohort size of CD-1 mouse		Number of infected mice in the cohort		Log killed trial (log-units)
		A	B	A	B	A	B	
750.3	18	200	2,000	5	5	2	5	0.7
750.4	18	1,000	10,000	5	5	4	5	0.8
751.1	18	1,000	10,000	5	5	3	5	1.1
751.2	18	50,000	500,000	5	5	2	5	3.1
751.3	18	90,000	900,000	5	5	0	3	4.1
752.1	18	1,000	10,000	5	5	2	4	1.6
752.3	18	80,000	800,000	5	5	4	5	2.7
753.1	18	50	500	5	5	2	5	0.1
753.2	18	50	500	5	5	2	4	0.3
754.2	18	50	500	5	5	3	5	-0.2
776.2	18	40	400	5	4	3	3	0.1
796.2	18	400	4,000	5	5	3	4	1.0
797.2	18	40	400	5	5	2	4	0.2
806.1	18	40	400	5	5	0	3	0.7
806.2	18	40	400	5	5	0	3	0.7
814.1	18	40	400	5	5	2	3	0.3
814.2	18	100	1,000	5	5	4	4	0.3
815.1	18	40	400	5	4	1	4	0.3
815.2	18	40	400	4	5	1	5	0.2
816.2	18	40	400	8	7	3	7	0.0
816.3	18	40	400	5	5	4	5	-0.6
819.2	18	40	400	5	5	4	4	-0.1
823.1	18	40	400	5	5	0	4	0.4
824.1	18	40	400	5	5	3	5	-0.3
826.2	18	40	400	5	5	4	4	-0.1